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FURTHER STUDIES OF THE INHERITANCE OF "ROGUE" TYPE IN GARDEN PEAS (PISUM SATIVUM L.)[1]

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INTRODUCTION

Since the rediscovery of Mendel's classical paper on heredity, characters of the garden pea (*Pisum sativum*) have been used to illustrate typical Mendelian inheritance. In 1915 Bateson and Pellew (*1*)[2] published an account of the genetics of types of peas known as "rogues"—types in which heredity has thus far appeared to be non-Mendelian. This paper was followed by later publications (*2*, *3*) dealing especially with variations in the proportion of rogues obtained from seeds from different parts of the same intergrading intermediate plant. Briefly stated, the facts reported were as follows:

(*a*) Certain varieties of the garden pea, such as Early Giant, Ne Plus Ultra, and Duke of Albany, are characterized by broad, wavy stipules and leaflets, both having rounded, emarginate apices and broad, straight pods. Occasionally there occur in pure-lines of such varieties plants called "rogues" that are described as "wild" or "vetch-like." The rogues differ from the parent form mainly in the reduction in width of the foliar parts (stipules and leaflets) and of the pods. The stipules of the rogues are narrow and pointed; the pods are narrow and curved along the upper suture. Rogues are not produced with any definite regularity or in such numbers that a characteristic ratio can be established between the rogues and parent form.

(*b*) Between the parent types and the vetch-like rogue are found intermediate forms, especially in the variety Early Giant. The intermediates are of several classes and may grade almost insensibly into types or rogues. The most usual form is characterized by broad foliage in the early stages of development, but as the plant matures the stipules become pointed and more roguelike. Such a plant is called by Bateson and Pellew an intergrading intermediate. Genetically there are two kinds of intermediates: (*1*) Those whose progeny consists of many rogues and few intermediates; and (*2*) those whose progeny consists of few rogues and many intermediates. The rogues from both kinds of families breed true, while the intermediates tend to produce rogues and intermediates in proportions comparable to those of their parents, i. e., there are high and low rogue-producing strains. Nevertheless, no regular ratio of rogues to intermediates can be found in successive generations.

[1] Accepted for publication Jan. 18, 1923. This contribution records cooperative work between the Bureau of Plant Industry and the University of Michigan which was carried on at the Department of Botany, University of Michigan.

[2] Reference is made by number (italic) to "Literature cited," p. 852.

(*c*) Any zygote derived from the fusion of a type gamete with a rogue gamete, whichever way the cross is made, produces a rogue; an intermediate gamete on union with a type gamete produces an intermediate or nonrogue form.

(*d*) Rogues are apparently produced more often from the upper (roguelike) portion of the intermediate plants than from the lower (typelike) part. It is also found that pollen grains produce a greater ratio of rogue gametes to nonrogue gametes than do the ovules, and that the proportion of rogue gametes produced by the pollen grains increases more rapidly, in going from the lower to upper flowers, than the proportion of rogue gametes produced by ovules.

(*e*) Intergrading intermediates of strains not throwing many rogues were found to produce rogues no more often from pods of the upper parts of the plant than from the lower nodes.

(*f*) Rogues with narrow, pointed stipules and narrow, curved pods, from whatever source, breed true.

(*g*) The cross rogue × type, no matter how made, produces only rogues in the F_1 and succeeding generations. In the seedling stage of the F_1 hybrid until the seventh or eighth node is developed, many of the plants are typelike in the character of the stipules and leaflets. These plants at maturity, with few exceptions, are all rogues. The plants in the F_2 generation are recognizable as rogues at all stages of development. There is apparently no segregation in the F_1 hybrids, at sporogenesis, of the rogue and type factors.

(*h*) Intervarietal crosses of rogue with type plants of varieties producing rogues give results similar to crosses of rogue with the variety from which the rogue originated as far as the rogue characters are concerned. Side by side with the apparently non-Mendelian behavior of the rogue characters is found the expected Mendelian segregation of such characters as pod shape, color of seed, shape of seed, and height of plant.

Bateson and Pellew (1) have suggested in explanation of the anomalous genetic behavior of the rogue characters that they are due to somatic segregation of the type "elements" in the F_1 hybrid so that the type "elements" are missing from the germ plasm at the time of gamete formation. Intergrading intermediates are assumed to be mosaics of type and rogue tissue, rogue gametes being formed from rogue tissue and type gametes from type tissue.

The present paper is in part a confirmation of the above results. However, a different interpretation of the results is advanced. In addition, data are submitted of the F_1, F_2, and F_3 generations of intervarietal crosses of rogue and type plants with type plants of varieties that apparently do not produce rogues. These crosses have given entirely new results, from which important conclusions have been drawn.

EXPERIMENTAL METHOD

The experimental methods used in the work here reported were the same as those described in a previous paper (*4*). At that time the fact of cross-pollination of rogue flowers by bumblebees was noted. In growing large cultures of peas it is impossible to protect all the individual flowers with paper or cloth coverings. In 1921 an attempt was made to grow peas under wire mosquito netting. This method is too expensive to be used on a large scale, so that in the future it seems advisable to carry on as much as possible of the breeding work with rogues in the greenhouse during the winter.

The results of 1919 and 1920 may be used to illustrate the amount of volunteer crossing that may be expected in the field in crosses between Gradus and Rogue. The number of volunteer hybrids is sufficiently great to make necessary some sort of protection of the rogue plants.

In 1919, 2,932 F_2 plants of the crosses Gradus rogue with Gradus (Table II) were grown. Of the 2,932 plants, 65, or 2.3 per cent, were volunteer crosses made by insects in 1918. The out-crosses were detected by the appearance of such dominant Mendelian characters as smooth seed, blunt pods, and colored flowers in F_2 cultures that should have contained only recessives for the three characters mentioned. Any crosses inter se, or out-crosses with recessive strains, could not be detected, but, apart from a few plants of Early Giant and of Peter Pan, no varieties which did not exhibit one of the three dominant characters were planted in the experimental garden in 1918. In 1920, 3,475 F_2 and F_3 plants of the cross rogue with type (Tables II, III and IV) were raised. Among them were 42 plants of obviously foreign origin, or about 1.2 per cent, showing that the amount of volunteer crossing was considerably less in 1919 than in 1918.

Occasional volunteer crosses have been found among the rogues and intermediate rogues, but no count has been kept. However, only one case of cross-pollination by insects has been noted among the thousands of typical Gradus plants grown.

Plants used in crosses have been previously inbred for at least one generation. The rogues used were either picked from commercial plantings of Gradus or arose de novo in pure cultures of Gradus plants. In either case the rogue strains were identical and gave the same results in crosses.

Uncontrolled cross-pollination in Pisum on the scale reported here was unlooked for at the beginning of these experiments and no steps were taken to insure self-pollination. By the time it was realized that rogues were an exception to the general rule that self-pollination is invariable in Pisum it was too late to protect the hybrid rogue plants in 1919. Aside from the writer's own experience with self-pollinating varieties of peas, his garden technic was of course influenced by the general conclusion of geneticists and practical horticulturists that peas are normally self-pollinated. White (*15*) found no cross with peas having yellow cotyledons among 10,000 seeds with green cotyledons grown at the Brooklyn botanic garden.

BEHAVIOR OF GRADUS ROGUES WHEN CROSSED WITHIN THE GRADUS STRAIN

F_1 GENERATION OF CROSSES BETWEEN GRADUS AND GRADUS ROGUE

In a former publication (*4*) the results in the F_1 generation of crossing typical Gradus with Gradus rogue [3] are reported.

The F_1 seedlings were variable in regard to stipule shape, showing gradations from typelike to roguelike plants. At maturity with one exception the F_1 plants were all rogues.

In addition to the hybrids between rogue and type previously listed by the author (*4*), 134 F_1 plants from 37 other crosses were grown, making a total from all sources of 282 F_1 plants of the cross Gradus × Gradus rogue, and reciprocal. Of the 282 plants all but three were classified as rogues at maturity.

[3] The name "Gradus rogue" or "Rogue" as used throughout this paper refers to the same type of mutation as that previously referred to (*4*) as a "Rabbit-ear" Rogue.

Two of the exceptional plants occurred in a single F_1 culture,[4] of which two plants were thorough rogues, while two others were intergrading intermediates, i. e., they were typelike at the lower nodes, but at upper nodes the stipules were pointed but much broader than in typical rogues. In the F_2 generation the progeny of the intergrading intermediates consisted of 32 and 33 rogues, respectively.

The third exceptional plant among the F_1 of rogue × type was described in 1919 (*4*). Its offspring in 1919 consisted of 19 intergrading intermediates and 15 type plants. The same cross and its reciprocal produced 7 other F_1 plants, described as rogues at maturity. From the character of the F_2 progenies (Table I) it is probable that the F_1 plants were in reality extreme intergrading intermediates, as the F_2 is characteristic of the progeny of intergrading intermediates. The plants behaved as intergrading intermediates of the sort described by Bateson and Pellew (*1*) as producing types, intergrading intermediates, and few or no rogues.

TABLE I.—*F_1 and F_2 generation of atypical hybrids of Gradus rogue × Gradus type and reciprocal*

1919 culture No.	Pedigree.	F_2		
		Rogues.	Intergrading intermediates.	Types.
9.1207	(G13–1–6–1t×G19–1–3r)—1	0	27	0
9.1208	(G13–1–6–1t×G19–1–3r)—2	0	23	0
9.1209	(G13–1–6–1t×G19–1–3r)—3	0	9	2
9.1210	(G13–1–6–1t×G19–1–3r)—4	0	0	23
9.1216	(G19–1–3r×G13–1–6–1t)—1	2	8	3
9.1217	(G19–1–3r×G13–1–6–1t)—2	0	27	0
9.1218	(G19–1–3r×G13–1–6–1t)—3	0	0	4
9.1219	(G19–1–3r×G13–1–6–1t)—4..Type foliage	0	19	15

F_2 AND F_3 GENERATIONS OF CROSSES BETWEEN GRADUS AND GRADUS ROGUE

From 184 F_1 hybrids of Gradus × Gradus rogue and reciprocal 4,319 F_2 plants were raised in 1919 and 1920. In the seedling stage the greater portion were easily recognizable as rogues; others could be termed intermediates, but none showed the typelike appearance found among the F_1 seedlings. (Among the 4,319 plants shown in Table II, 81, or 1.8 per cent, were from foreign pollination, as explained elsewhere in this paper. The volunteer out-crosses were generally distinguished in the seedling stage as plants with typelike stipules.) At maturity the plants were either rogues or various sorts of intermediates. Thirty-nine F_2 rogues gave in the F_3 generation 575 plants, all rogues except five plants (0.8 per cent). The products of volunteer out-crossing in 1919 are shown in Table III.

[4] The intergrading intermediates were plants No. 3 and No. 4 of cross (G10–1–12–1t×G23–1–1–16r) in the F_1 generation.

TABLE II *a*.—*Gradus rogue* × *Gradus type and reciprocal in the* F_2 *generation*

Culture No.	Rogues.	Crosses.
1919:		
9.1200	10	
9.1201	3	
9.1202	13	
9.1203	16	
9.1204	4	
9.1205	10	
9.1206	3	
9.1211	29	
9.1212	39	
9.1213	36	
9.1214	35	
9.1215	54	2
9.1220	4	
9.1221	4	
9.1222	2	
9.1223	2	
9.1226	12	
9.1227	14	
9.1251	80	
9.1252	82	
9.1253	86	
9.1254	10	
9.1255	208	
9.1265	37	3
9.1266	27	
9.1267	24	
9.1268	51	1
9.1269	15	1
9.1270	37	
9.1271	39	
9.1272	47	
9.1273	28	
9.1274	27	3
9.1275	34	4
9.1276	57	3
9.1277	54	
9.1278	43	1
9.1279	6	
9.1280	19	
9.1284	118	4
9.1285	73	1
9.1286	36	
9.1287	49	1
9.1288	85	2
9.1289	22	1
9.1293	6	1
9.1294	52	4
9.1295	73	12
9.1296	54	
9.1297	19	
9.1298	17	2
9.1299	43	1
9.1300	32	
9.1301	95	
9.1302	77	7
9.1304	45	1
9.1303	15	
9.1305	71	1
9.1306	46	
1919—Continued.		
9.1307	29	
9.1308	18	
9.1309	25	
9.1316	58	2
9.1317	13	3
9.1318	44	
9.1319	73	
9.1320	108	4
9.1321	31	
9.1322	43	
9.1323	4	
9.1324	21	
9.1325	25	
9.1326	46	
Total	2,867	65
1920:		
0.89	15	2
0.90	17	1
0.91	12	
0.92	37	1
0.93	12	
0.94	7	
0.95	15	1
0.96	7	
0.97	30	
0.98	48	1
0.99	10	
0.100	6	
0.101	14	
0.102	7	
0.103	15	
0.104	14	1
0.105	14	
0.106	12	
0.107	30	
0.108	26	
0.109	10	
0.110	9	
0.112	21	2
0.113	20	1
0.114	13	
0.115	8	
0.116	18	
0.117	18	
0.118	15	
0.119	20	
0.120	22	
0.121	21	
0.122	18	
0.123	31	
0.124	12	
0.125	25	
0.126	14	
0.127	25	
0.128	22	
0.129	16	
0.130	15	

a In Tables II, III, and IV, unless otherwise noted, the parent plants were rogues in each instance. The column headed "Crosses" shows the number of volunteer hybrids occurring in the various cultures.

TABLE II.—*Gradus rogue X Gradus type and reciprocal in the F_2 generation*—Continued.

Culture No.	Rogues.	Crosses.	Culture No.	Rogues.	Crosses.
1920—Continued.			1920—Continued.		
0.131	36	.....	0.199	9	1
0.184	27	.....	0.200	32	.....
0.185	20	.....	0.202	6	.....
0.186	23	.....	0.203	15	.....
0.187	38	.....	0.204	18	.....
0.188	37	.....	0.205	30	.....
0.189	34	.....	0.206	37	2
0.190	8	.....	0.207	3	.....
0.191	26	2	0.208	14	.....
0.192	10	.....	0.209	39	.....
0.193	26	.....	0.210	36	.....
0.194	21	.....	0.211	7	.....
0.195	31	.....	0.212	2	.....
0.196	29	.....	0.213	32	.....
0.197	7	.....			
0.198	21	1	Total (1919-20)	4,238	81

TABLE III.—*Gradus rogue × Gradus type and reciprocal in the F_3 generation*

Culture No.	Rogues.	Crosses.	Culture No.	Rogues.	Crosses.
1920:			1920—Continued.		
0.371	22	.....	0.391	10	.....
0.372	10	.....	0.392	15	.....
0.373	23	.....	0.393	12	.....
0.374	12	.....	0.394	14	.....
0.375	11	.....	0.395	16	.....
0.376	13	.....	0.396	24	2
0.377	16	.....	0.397	3	.....
0.378	13	.....	0.398	25	.....
0.379	3	.....	0.399	17	1
0.380	6	.....	0.400	21	.....
0.381	6	.....	0.401	18	.....
0.382	15	.....	0.402	11	.....
0.383	10	.....	0.403	22	.....
0.384	20	.....	0.404	17	.....
0.385	12	.....	0.405	18	.....
0.386	13	.....	0.406	23	.....
0.387	17	.....	0.407	23	2
0.388	17	.....	0.408	18	.....
0.389	15	.....			
0.390	14	.....	Total	575	5

F_1 AND F_2 GENERATIONS OF CROSSES BETWEEN GRADUS TYPE AND THE F_1 OF GRADUS TYPE WITH GRADUS ROGUE

The cross type × (type × rogue) gave an F_1 of 88 plants which showed a wide range of variation from typelike to roguelike plants in the seedling stage, but which at maturity were all rogues. Sixty-six F_1 plants of the sesquireciprocal crosses gave an F_2 progeny (Table IV) of 1,477 rogues, 6 intermediate types, and 22 volunteer hybrids (1.4 per cent).

The exceptional intermediate types occurred in the F_2 culture 0.322, which also contained 23 rogues and 1 out-crossed plant. Seven other families from the original cross which produced culture 0.322 gave only rogues in the F_2 generation.

TABLE IV.—*Back-crosses Gradus type×(Gradus type×Gradus rogue) in F_2 generation*

Culture No.	Rogues.	Others.	Crosses.	Culture No.	Rogues.	Others.	Crosses.
1920:				1920—Contd.			
0.216	3			0.325	23		
0.217	2			0.326	16		1
0.292	36		2	0.327	22		
0.293	25			0.328	17		
0.294	23			0.329	40		
0.295	23			0.330	31		
0.296	2			0.331	41		
0.297	31			0.333	18		
0.298	18			0.334	54		1
0.299	11			0.335	22		
0.300	11			0.336	36		
0.301	38			0.338	73		
0.302	12			0.341	18		1
0.303	27			0.342	21		
0.304	1			0.343	20		2
0.305	6			0.344	6		
0.306	27			0.345	28		
0.307	17			0.346	20		
0.308	20		1	0.347	15		
0.309	20			0.348	23		
0.310	16			0.349	18		
0.311	44		4	0.350	23		
0.312	33			0.351	15		
0.313	17			0.352	30		
0.314	10			0.353	26		
0.315	44			0.355	7		
0.316	45		2	0.356	13		
0.317	38		2	0.357	18		5
0.318	7			0.358	27		
0.319	16			0.368	14		
0.320	31			0.369	22		
0.321	3			0.370	15		
0.322	23	6	1				
0.324	35			Total	1,487	6	22

STATISTICAL STUDY OF THE INHERITANCE OF STIPULE SHAPE IN GRADUS ROGUE AND GRADUS TYPE

In order to get a more exact comparison of the rogues and types, on the one hand, and the F_1 and F_2 generations of hybrids between the two, measurements of the length and width of the stipules from the fourth to the ninth node were made in the various categories of plants. The ratio of width to length of stipule was then calculated. Because of disease and mechanical injury the same number of stipules could not be measured at each node in each group of plants. The class frequency distributions (Table V) are, therefore, expressed as percentages of the total number of variates. In this manner 49 pure type plants, 43 pure rogues, 69 F_1 hybrids, and 104 F_2 plants were measured.

The mean, standard deviation, and coefficient of variation of the ratios at each node and for the plant as a whole were calculated from the data in Table V. These statistical constants are given in Table VI. Figure 1 shows the graphs obtained by plotting the means for each node in each

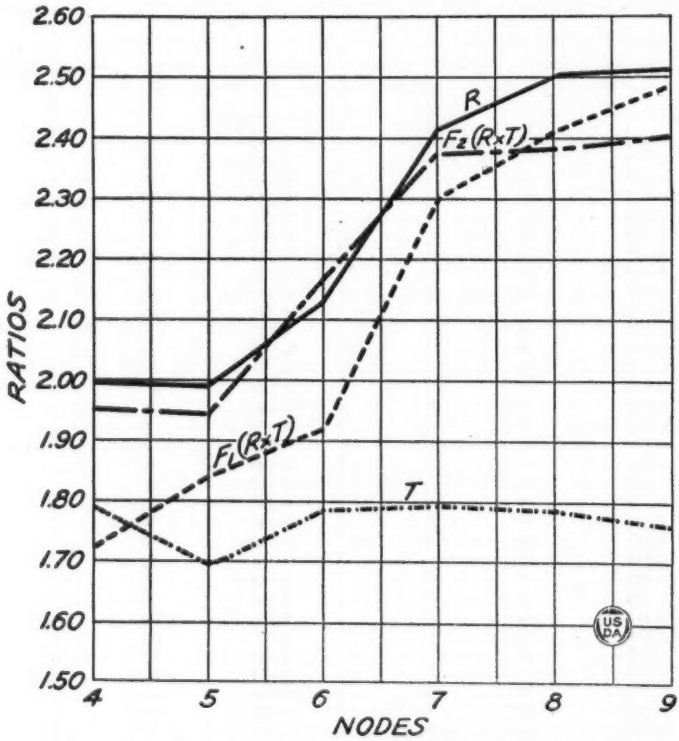

FIG. 1.—Graphs obtained by plotting the mean ratio of $\frac{\text{length of stipule}}{\text{width of stipule}}$ at the fourth to ninth nodes of Gradus type (T), Rogue (R), and the F_1 and F_2 hybrids of crosses between Gradus and Gradus rogue, based on data from Table VI.

group of plants. The similarity of the F_1 and F_2 generations to the rogue parent is easily seen by inspection of Figure 1 and Tables V and VI. The F_1 hybrids up to the seventh node are like the type, or intermediate between the type and rogue; above the seventh node the plants become rapidly more roguelike. At all nodes the F_1 plants appear more variable than Gradus and are like the rogues in this respect.

TABLE V.—*Frequency distribution of ratio* $\left(\frac{\text{length of stipule}}{\text{width of stipule}}\right)$ *at the fourth to the ninth nodes in Gradus rogue, Gradus, F_1 and F_2 hybrid plants. As the number of stipules measured at each node was not always the same, the ratios in any one class are expressed in percentages*

Ratio.	Nodes.						
	Fourth.	Fifth.	Sixth.	Seventh.	Eighth.	Ninth.	Total.
Gradus rogue:	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1.25	.....	.....	.....	.....	.....	.....	.....
1.35	.....	.....	3.02	3.02	.....	.....	0.45
1.45	2.66	1.20	.....	0	.....	.....	.67
1.55	5.32	2.40	1.16	0	.....	.....	1.56
1.65	10.64	6.00	1.16	1.51	.....	.....	3.36
1.75	13.30	8.40	3.48	0	.....	3.56	4.93
1.85	9.31	18.00	9.28	0	3.75	3.56	7.84
1.95	5.32	12.00	10.44	3.02	0	1.78	5.79
2.05	11.97	25.20	24.36	4.53	2.50	0	12.55
2.15	10.64	8.40	10.16	4.53	2.50	0	6.73
2.25	15.96	8.40	16.24	10.35	6.25	5.34	10.76
2.35	6.65	4.80	10.16	12.08	16.25	5.34	9.64
2.45	5.32	3.60	.48	21.14	12.50	1.24	9.42
2.55	1.33	1.20	4.64	16.61	21.25	28.44	11.21
2.65	0	.....	1.16	19.63	16.25	19.58	8.52
2.75	1.33	.....	0	3.02	10.00	8.90	3.58
2.85	.....	.....	1.16	.....	0	5.34	.89
2.95	.....	.....	.....	.....	6.25	1.78	1.34
3.05	.....	.....	.....	.....	1.25	1.78	.45
3.15	.....	.....	.....	.....	1.25	.....	.22
	[a] 75	[a] 83	[a] 86	[a] 66	[a] 80	[a] 56	.....
Gradus:							
1.25	.....	.....	.....	.....	.....	.....	.....
1.35	5.40	1.08	2.24	.....	.....	.....	1.59
1.45	6.48	3.24	3.36	6.18	1.08	.....	3.78
1.55	10.80	19.44	8.96	10.30	7.56	9.52	11.35
1.65	11.88	24.84	11.20	13.39	15.12	21.42	15.93
1.75	19.44	23.76	30.24	19.57	29.16	33.32	25.30
1.85	35.64	18.36	20.16	23.69	27.00	19.04	24.70
1.95	3.24	7.56	13.44	13.39	16.20	10.90	10.95
2.05	4.32	0	6.72	7.21	2.16	4.76	4.18
2.15	0	1.08	3.36	6.18	0	0	.20
2.25	0	0	0	0	0	0	0
2.35	1.08	.....	.....	.....	.....	.....	.02
2.45	.....	.....	.....	.....	.....	.....	.....
	[a] 91	[a] 92	[a] 89	[a] 97	[a] 91	[a] 42	.....
F_1 hybrid:							
1.25	.85	.....	1.46	.....	.....	.....	.41
1.35	.85	.75	0	.77	.....	.....	.41
1.45	5.10	1.50	1.46	0	.....	.....	1.38
1.55	16.15	10.50	1.46	.77	.....	.....	4.98
1.65	30.60	16.50	10.22	.77	.....	.....	10.11
1.75	22.10	16.50	10.95	.77	1.60	.....	9.14
1.85	22.10	19.50	20.44	4.62	1.60	.....	12.18
1.95	4.25	10.50	13.14	5.39	1.60	.....	6.37
2.05	3.40	18.75	12.41	10.01	8.80	1.35	8.44
2.15	0	12.00	9.49	11.55	3.20	1.35	5.67
2.25	.85	9.00	10.95	13.86	12.00	10.80	8.72
2.35	1.70	.75	3.65	17.71	12.00	20.25	8.44
2.45	.....	0	.73	8.47	17.60	18.90	6.65
2.55	.....	0	2.19	14.63	19.20	24.30	8.86
2.65	.....	.75	.73	6.16	10.40	14.85	4.71
2.75	.....	.....	.....	2.31	7.20	5.40	2.21
2.85	.....	.....	.....	1.54	.80	1.35	.55
2.95	.....	.....	.....	.....	3.20	1.35	.69
	[a] 117	[a] 132	[a] 136	[a] 129	[a] 124	[a] 74	.....

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TABLE V.—*Frequency distribution of ratio* $\left(\frac{\text{length of stipule}}{\text{width of stipule}}\right)$ *at the fourth to the ninth nodes in Gradus rogue, Gradus F_1 and F_2, hybrid plants. As the number of stipules measured at each node was not always the same, the ratios in any one class are expressed in percentages*—Continued.

Ratio.	Nodes.						
	Fourth.	Fifth.	Sixth.	Seventh.	Eighth.	Ninth.	Total.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
F_2 hybrid:							
1.05	.49	.....	.....	.....	.....	.....	.08
1.15	.96	.49	.....	.....	.....	.....	.24
1.25	.96	0	.....	.....	.....	.....	.16
1.35	.96	.49	.....	.....	.....	.....	.24
1.45	.48	.49	.....	.....	.....	.....	.16
1.55	1.45	.98	.....	.....	.....	.....	.41
1.65	6.26	3.42	.....	.....	.....	.....	1.62
1.75	11.20	14.20	.96	.....	.....	.....	4.37
1.85	15.80	22.42	4.33	1.44	.49	.49	7.52
1.95	14.50	20.42	3.85	1.44	.49	.49	6.87
2.05	26.10	21.40	27.40	4.82	4.90	2.94	14.63
2.15	8.20	6.34	21.61	8.18	8.78	5.88	9.70
2.25	8.67	3.42	11.52	16.33	17.55	15.70	13.90
2.35	1.45	3.90	5.30	26.40	20.00	25.00	14.70
2.45	.96	.49	2.88	14.90	17.55	22.10	10.20
2.55	.48	.49	.48	18.20	21.92	13.20	9.55
2.65	.96	0	.....	4.81	4.39	10.30	3.48
2.75	.48	.49	.....	2.40	3.42	2.40	1.53
2.85	.....	.....	.....	.96	.49	1.47	.48
	[a] 207	[a] 205	[a] 208	[a] 208	[a] 205	[a] 204	.....

[a] The actual number of measurements from which the frequency distributions (expressed in percentages) were derived.

TABLE VI.—*Constants for stipule shape calculated for each node separately and for the plant as a whole, based on data in Table V*

	Nodes.						
	Fourth.	Fifth.	Sixth.	Seventh.	Eighth.	Ninth.	All.
Type:							
M	1.705	1.706	1.782	1.797	1.782	1.766	1.765
σ	.205	.155	.170	.177	.130	.130	1.53
C. V.	12.05	8.11	9.55	9.88	7.30	7.32	8.66
Rogue:							
M	2.001	1.994	2.133	2.411	2.500	2.515	2.259
σ	.294	.223	.223	.288	.257	.266	.339
C. V.	14.70	11.20	10.46	11.53	10.28	10.59	15.00
F_1 hybrid:							
M	1.725	1.846	1.926	2.308	2.410	2.485	2.118
σ	.174	.217	.275	.266	.261	.172	.377
C. V.	10.11	11.79	14.32	11.56	10.82	6.93	17.79
F_2 hybrid:							
M	1.957	1.941	2.173	2.370	2.384	2.405	2.205
σ	.256	.206	.148	.184	.170	.171	.275
C. V.	13.08	10.61	6.81	7.75	7.13	7.71	12.47

The F_2 hybrids differ from the F_1 generation in being remarkably like the pure rogues at all stages in their development. The stipules at the fourth, fifth, and sixth nodes of the former are especially different from those of the F_1 plants. The measurements serve to emphasize what is plainly seen by inspection of the cultures, namely, that the F_2 seedlings resemble the pure rogues of the same age rather than the young F_1 plants which so generally exhibit a typelike character. To the eye, neither of the hybrid generations, at maturity, differs in any appreciable way from the rogue parent.

Besides measuring the stipules at the fourth to the ninth nodes in the case of the rogue, type, and F_1 and F_2 hybrids, measurements were made of four stipules per plant from about the eighth to the fourteenth node of mature individuals in each category. The mature plants measured included those used in the comparison by nodes of the four groups, and, in the case of all but the F_1 hybrids, additional plants were measured.

In Table VII are shown the frequency distributions and statistical constants calculated for each of the four categories of plants. The range of variation is strikingly significant, the range of the type not overlapping that of the rogue. The F_1 and F_2 hybrids have a range of variation equal in extent to that of the rogue parent. The F_2 generation differs from the pure rogue and the F_1 in that it is less variable as indicated by the coefficients of variation (Coefficient Variation): F_2 generation, 5.84 ± 0.2677; rogue, 7.06 ± 0.2912; F_1 generation, 7.09 ± 0.3662, respectively. The probable errors of the differences between the coefficients of variation of the pure rogue and of the F_2, and of the F_2 and F_1, are practically three times the difference, in each case indicating that the differences are real. However, the differences in variability of the three groups as shown by the stipule measurements of the mature plant were not apparent on inspection of the cultures.

The resemblance of the F_1 and F_2 hybrids to the pure rogues is further accentuated by a comparison of the mean stipule ratio of the three. The mean for the rogue parent is 2.339 ± 0.0096; for the F_1, 2.383 ± 0.0196; and for the F_2 it is 2.360 ± 0.0089. There is no significant difference between any of the three means.

Thus in regard to variability, range of variation, and mean stipule ratio, the two hybrid generations resemble only the rogue parent. The influence of the type parent, in crosses between type and rogue is apparent only in the F_1 plant and at only the lower nodes.

CROSSES BETWEEN THE GRADUS STRAIN AND MUMMY, A NONROGUE-PRODUCING VARIETY.

In 1918 the English "Mummy" pea was used in crosses with Gradus rogue and Gradus type. Seed of the Mummy variety was first obtained from O. E. White of the Brooklyn Botanical Garden (White's No. P1–3–4–2), and only this strain has been used in the crosses. In Mummy, fasciation of the main stem is characteristic, but does not appear until the plant is well developed. With the appearance of the fasciated condition the stipules become narrower and more pointed than those at the lower and middle nodes of the same plant. Plate 2, A, B, C is of a mature plant of Mummy. Although having stipules much narrower than in Gradus type, no rogue comparable to those found in Gradus have ever been observed by the writer among the several thousand

TABLE VII.—*Frequency distributions and statistical constants of ratio of* $\frac{\text{length of stipule}}{\text{width of stipule}}$ *for Gradus type, Gradus rogue, P 16, and of the* F_1 *and* F_2 *generations of various hybrids and hybrid segregates from crosses between the three parent types. The frequency distributions and statistical constants for the* F_2 *generation of Gradus rogue×Mummy are based on data in Table XIV.*

Types, hybrids and segregates.	1.35	1.45	1.55	1.65	1.75	1.85	1.95	2.05	2.15	2.25	2.35	2.45	2.55	2.65	2.75	2.85	2.95	3.05	Total.	M	σ	Coefficient variation.
Gradus type (P_1)	1	0	16	64	33	18	3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	135	1.693±0.0107	0.098±0.0075	5.78±0.2381
Gradus rogue (P_1)	.....	.....	.....	.....	.....	.....	.....	7	21	33	30	22	10	8	4	.....	.....	.....	135	2.339±.0096	.165±.0068	7.06±.2912
Gradus type×Gradus rogue (F_1)	.....	.....	.....	.....	.....	.....	.....	6	4	18	23	12	11	10	2	.....	.....	.....	86	2.383±.0196	.169±.0087	7.09±.3662
Gradus type×Gradus rogue (F_2)	.....	.....	.....	.....	.....	.....	.....	4	3	33	30	23	10	5	1	.....	.....	.....	109	2.360±.0089	.138±.0063	5.84±.2677
Mummy (P_1)	.....	.....	.....	.....	.....	3	29	68	56	15	5	.....	.....	.....	.....	.....	.....	.....	176	2.088±.0050	.100±.0073	4.77±.1719
Gradus type×Mummy (F_2), all	.....	1	1	7	21	19	24	8	2	1	2	.....	.....	.....	.....	.....	.....	.....	87	1.868±.0115	.159±.0081	8.51±.4382
AA and *Aa* segregates (colored)	.....	.....	.....	3	12	15	11	8	2	0	2	.....	.....	.....	.....	.....	.....	.....	53	1.905±.0142	.154±.0101	8.08±.5394
A, B, C, segregates (purple)	.....	.....	.....	2	9	10	8	6	2	0	2	.....	.....	.....	.....	.....	.....	.....	39	1.909±.0175	.162±.0124	8.48±.6523
A, B, segregates (pink)	.....	.....	.....	1	3	5	3	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	14	1.864±.1950	.112±.0136	6.00±.7415
aa, segregates (white)	.....	1	1	4	6	3	6	0	0	1	.....	.....	.....	.....	.....	.....	.....	.....	22	1.710±.0260	.181±.0184	10.58±1.0873
Gradus rogue×Mummy (F_2) all	.....	1	1	11	37	86	143	167	122	136	187	231	169	131	58	39	12	4	1,534	2.307±.0049	.288±.0035	12.48±.1542
AA, Aa, segregates (colored)	.....	.....	.....	4	18	52	107	133	88	100	152	181	142	107	46	33	11	4	1,178	2.326±.0016	.281±.0011	12.08±.0475
A, B, C, segregates (purple)	.....	.....	.....	4	12	40	79	95	66	78	118	132	106	79	29	24	9	2	873	2.325±.0063	.278±.0045	11.95±.1805
A, B, c, segregates (pink)	.....	.....	.....	.....	6	12	28	38	22	22	34	49	36	28	17	9	2	2	305	2.336±.0110	.286±.0078	12.24±.3392
aa, segregates (white)	.....	.....	1	7	19	34	36	34	34	36	35	50	27	24	12	6	1	.....	356	2.230±.0106	.297±.0075	13.31±.3338
Gradus rogue×Mummy (F_2) all (omitting Nos. 0.1059 and 0.1093)	.....	.....	1	8	30	72	102	122	110	133	183	230	169	131	58	39	12	4	1,404	2.333±.0050	.280±.0036	12.00±.1549
AA and *Aa,* segregates (colored)	.....	.....	.....	3	16	45	74	94	77	97	148	180	144	107	46	33	11	4	1,077	2.355±.0056	.275±.0039	11.67±.1720
A, B, C, segregates (purple)	.....	.....	.....	3	12	37	58	64	60	75	115	132	106	79	29	24	9	2	805	2.349±.0064	.271±.0045	11.53±.1964
A, B, c, segregates (pink)	.....	.....	.....	.....	4	8	16	30	17	22	33	48	36	28	17	9	2	2	272	2.375±.0110	.269±.0078	11.32±.3427
aa, segregates (white)	.....	.....	1	5	14	27	28	28	33	36	35	50	27	24	12	6	1	.....	327	2.258±.0110	.294±.0077	13.02±.3491
Gradus rogue×Mummy (F_2) all (Nos. 0.1059 and 0.1093)	.....	.....	.....	3	7	14	41	45	12	3	4	1	.....	.....	.....	.....	.....	.....	130	1.998±.0082	.139±.0058	6.95±.2912
AA and *Aa* segregates (colored)	.....	.....	.....	1	2	7	33	39	11	3	4	1	.....	.....	.....	.....	.....	.....	101	2.027±.0087	.127±.0060	6.26±.2982
A, B, C, segregates (purple)	.....	.....	.....	1	0	3	21	31	6	3	3	.....	.....	.....	.....	.....	.....	.....	68	2.038±.0095	.116±.0067	5.69±.3290
A, B, c, segregates (pink)	.....	.....	.....	.....	2	4	12	8	5	0	1	1	.....	.....	.....	.....	.....	.....	33	2.008±.0170	.145±.0120	7.22±.6025
aa, segregates (white)	.....	.....	.....	2	5	7	8	6	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	29	1.899±.0159	.127±.0112	6.68±.5899

plants of Mummy grown in the last three years. The Mummy variety differs from Gradus in several Mendelian characters.

NOMENCLATURE.—The factors concerned in the crosses of Mummy and Gradus and the symbols used to designate the factors are as follows:

A. Factor for flower color. A must be present to have any color develop. Alone A produces light purple flowers; a produces white flowers. Dominance is complete. Tedin, (*11*).
B. Interacts with A to produce pink flowers; b has no visible effect. Tedin, (*11*).
C. Interacts with A to produce violet flowers. Interacts with AB to produce dark purple; c has no visible effect on A or B. Tedin, (*11*).
N. Factor for normal stem as opposed to n for fasciated. Mendel, (*8*); White, (*14*).
X. Factor for mean stipule ratio of approximately 2.35. The allelomorph x in Gradus determines a ratio of about 1.70. (Described here.)
x′. Factor for stipule shape in Mummy allelomorphic to X in Gradus rogue and x in Gradus.
Y. Factor responsible in Mummy for mean stipule ratio of approximately 2.05. Its allelomorphy in Gradus determines a stipule ratio of about 1.70. (Described here.)

It is tentatively assumed that the change which takes place when Gradus produces Rogue is a point mutation of factor x to X.

To find the mean stipule ratios for which it is assumed Xx and Yy are responsible, the average ratio of width to length was obtained for four stipules from the middle nodes of each plant measured. In this manner the mean stipule ratio per plant was obtained for 135 Gradus types, 135 Gradus rogues, and 176 plants of the Mummy variety. Frequency distributions of the three groups of mean ratios were made (Table VII) and the mean, standard deviation, and coefficient of variation calculated for each category of plants. The mean stipule ratios were found to be: Gradus type 1.693 ± 0.0107; Gradus rogue, 2.339 ± 0.0096; and Mummy, 2.088 ± 0.0050.

In regard to the factors here considered, the three strains have the following genetic constitution:

Gradus type, aaBBCCxxyyNN.
Gradus rogue, aaBBCCXXyyNN.
Mummy, AABBccx′x′YYnn.

GRADUS TYPE × MUMMY

RELATION OF THE X AND X′ FACTORS FOR STIPULE WIDTH

From the data at hand, factors x and x′ are considered to have practically the same somatic expression in regard to stipule shape. The difference is in the ability of x to mutate to X, a quality lacking in x′, at least in the x′x′ or xx′ combination. In other respects the two allelomorphic factors x and x′ can be viewed as essentially indentical, as far as visible expression is concerned.

RELATION OF COLOR FACTORS A AND a

Considering the allelomorphic pair A and a in the F_2 generation of crosses of Gradus type with Mummy, the colored flowered plants (AA and Aa) and noncolored (aa) are found to be in close agreement with the expected 75 and 25 per cent (Table VIII).

TABLE VIII.—*Gradus×Mummy and Gradus rogue×Mummy in the F_2 generation (aa×AA)*

TYPE×MUMMY

	AA, Aa.	aa.	Total.
Observed	563	179	[a] 742
Percentage found	75.88	24.12	.....
Percentage expected	75.00	25.00	.....
Difference (D)	0.88	0.88	.....
Standard error (S. E.)	1.17	.....	.....
D/S. E	0.75	.....	.....

ROGUE×MUMMY

Observed	1178	356	1,534
Percentage found	76.79	23.21	.....
Percentage expected	75.00	25.00	.....
Difference (D)	1.79	1.79	.....
Standard error (S. E.)	.74	.....	.....
D/S. E	2.41	.....	.....

[a] Data from 25 F_3 families including 2 families (34 plants) classified as to color segregation and measured for ratio of width to length of stipule but not classified as to stipule shape, i. e., as types or intermediates.

RELATION OF COLOR FACTORS A AND C

The F_1 plants of Gradus type×Mummy were all purple-flowered. The F_2 generation gave the expected recombinations in approximately the proportion 56.25 per cent purple (with factors A, B, and C), 18.75 per cent pink (with factors A and B), and 25.00 per cent white (with factors aa), the observed number in each class being in sufficient agreement with that theoretically expected when two factors are independently inherited (Table IX).

TABLE IX.—*Segregation of factors for flower color in F_2 generation of cross Gradus with Mummy and of Gradus rogue with Mummy (aaBBCC×AABBcc)*

GRADUS×MUMMY

	Purple ABC.	Pink ABc.	White aBc, aBC.	Total.
Observed	406	157	179	742
Percentage found	54.72	21.16	24.12	.....
Percentage expected	56.25	18.75	25.00	.....
Difference	1.53	2.41	.88	.....
χ^2=2.8854.				
P=.3405.				

GRADUS ROGUE×MUMMY

Observed	873	305	356	1,534
Percentage found	56.91	19.88	23.21	.....
Percentage expected	56.25	18.75	25.00	.....
Difference	.66	1.13	1.79	.....
χ^2=3.0225.				
P=0.2214.				

From seeds of 25 F_1 hybrids, 742 F_2 hybrids were matured in 1920. A total of 708 individuals, belonging to 23 families, were classified according to stipule shape as Graduslike types (yy) and intermediates (YY or yy), the latter class being made up of plants resembling Mummy in stipule character. The remaining families, consisting of 34 plants, were not directly classified in the same way, but the mean stipule ratio per plant was calculated from measurements of the width and length of the stipules. A record of the flower color was kept for all the 742 F_2 plants (Tables VIII and IX).

Of the 708 plants classified according to stipule shape, 540 were grouped as intermediates resembling Mummy and 168 as Graduslike types (Table X), indicating a single factor difference, in regard to stipule shape, between Gradus type and Mummy. The expected percentage in the two groups is 75 per cent intermediates and 25 per cent types; the observed percentages, 76.27 per cent intermediates and 23.73 per cent Graduslike types, in the F_2, are well within the limits of the standard error. The standard error as used here is calculated according to the formula of Yule (*16*).

TABLE X.—*Segregation of stipule shape in F_2 generation of Gradus×Mummy* (yy×YY)

GRADUS×MUMMY

	YY, Yy	yy	Total.
Observed	540	168	708
Percentage found	76.27	23.73	.....
Percentage expected	75.00	25.00	.....
Difference (D)	1.27	1.27	.....
Standard error (S. E.)	1.09	.....	.....
D/S.E.	1.16	.....	.....

RELATION OF Y AND y, FACTORS FOR STIPULE SHAPE

In all, 37F_1 plants were grown in 1919 from the cross Gradus type with Mummy. In size and shape the stipules of the F_1 plants were intermediate between Mummy and Gradus type. The upper part of a branch of a mature F_1 plant is shown in Plate 3, A.

LINKAGE RELATION OF Yy AND Aa

The relation of the factor pairs Yy and Aa was determined from a count of 708 F_2 plants of the cross Gradus type with Mummy (aayy×AAYY). In Table XI are tabulated the observed numbers of the F_2 recombinations. The actual ratios of the four classes AY, aY, Ay, and ay indicate that factors AY and ay are linked. The calculated zygotic series is approximately 66 AY : 9 Ay : 9 aY : 16 ay; the gametic series approximately 4 : 1 : : 1 : 4; and the percentage of crossing-over for all the F_2 families is 20.41 ± 1.28 per cent, using Haldane's method (*6*) for the determining of zygotic and gametic series from observed F_2 frequencies.

TABLE XI.—*Linkage relation of* Aa *and* Yy *from F_2 generation of Mummy×Gradus*

	aayy×AAYY.				
	AY.	Ay.	aY.	ay.	Total.
Observed	476	63	64	105	708
Calculated	467	64	64	113	.....
Difference	9	1	0	8	.....
$\chi^2 = 0.7554$	.....	.....	.....	.....	.....

Plates 4 A, B, and 5 A, B, are of the upper parts of a Graduslike type and of an intermediate segregate, respectively, from the F_2 of Gradus × Mummy. The intermediate shown has stipules wider than the stipules of the F_1 plant of the same cross shown in Plate 3, A. The difference is largely due to the fact that the stipules of the F_1 plant are on a branch while those of the F_2 intermediate are on the main stem. Stipules on a branch are smaller and narrower than those of the main stem in nearly all instances, irrespective of the variety or strain.

It should be pointed out here that Y and y are considered as the factors chiefly responsible for the difference for stipule shape between Mummy and Gradus. It is highly probable, from the increased variability of the F_2 generation over that of the parents, that a number of modifying factors interact with Y and y to affect stipule shape.

RELATION OF FACTORS Aa FOR FLOWER COLOR AND Nn FOR CHARACTER OF STEM

It has been said before that stipules from the fasciated portion of the stem of Mummy are narrower than those of the apparently nonfasciated part of the same plant or from a normal plant. The F_1 plants of the crosses of Mummy with Gradus were normal stemmed and had narrow, intermediate, or Mummylike stipules. No accurate counts were made of the fasciated F_2 segregates, but apparently the expected 3 : 1 ratio of normal to fasciated plants occurred.

Since fasciated plants appeared, on the whole, to have narrower stipules than normal plants, it might be suggested that linkage existed between n and A, and N and a, so that the existence of a factor pair Yy would not have to be assumed. That such is not the case is shown by the results of White (*14*), who found factors Aa and Nn to be independently inherited. The two cases are comparable as the AAnn parent of the hybrids studied by White was from the same strain as that used by the present writer.

STATISTICAL EVIDENCE OF LINKAGE BETWEEN FACTORS AY AND ay

In addition to the stipule measurements of the 34 plants mentioned in the preceding section, stipules of 41 other plants were measured from the F_2 generation of Gradus × Mummy. Of the 75 plants measured (Table VII), 53 were A segregates (39 purples and 14 pinks) and 23 were a segregates (whites). The mean stipule ratio of the F_2 plants as a whole was found to be 1.868 ± 0.0126, for the A's 1.905 ± 0.0142, and for the a's 1.710 ± 0.0260. Considering a difference between the means of at least three times the probable error of the difference as being significant, it is found that the difference between the means of the A and a segregates is significant, since it is five times the standard error.

The same relation holds between the mean ratio of the colored and white F_2 segregates, whether the A plants be considered collectively, or separately as purples (with the factors A and B) and as pinks (with the factors A and b), the mean of the purples being 1.909 ± 0.0175 and of the pinks 1.864 ± 0.0142 as compared with 1.905 ± 0.0142 for the A segregate as a whole (Table VII).

No essential difference exists between the means of Gradus and the white-flowered (aa) F_2 segregates from the cross Mummy with Gradus. However, the hybrid whites show a greater variability than do the pure whites, the coefficient of variation of the former being 10.58 ± 1.0870 compared with 5.78 ± 0.2381 for the latter.

The increased variation of the F_2 aa plants is probably due for the most part to crossing-over of the factors Yy for stipule shape and partly to the recombination of modifying factors affecting the same character.

The mean ratio of the A segregates, 1.905 ± 0.0142, is significantly less than that of the Mummy parent, 2.088 ± 0.0050, and the variability of the former is greater than that of the pure AA plants. The increased variability of the colored-flowering segregates, besides being accounted for largely by crossing-over, could, in addition, be due to a dissimilarity in stipule shape of AA and Aa plants. The heterozygous plants being in excess would tend to lower the mean of the population. Actually the F_1 plants did appear to have stipules intermediate in shape between those of the two parents, but no measurements are available for comparison.

It is realized that the number of F_2 individuals measured is small but, taken alone, the statistical results would offer fairly good evidence of linkage between the factor or factors for stipule shape and the A factor for flower color. In connection with the F_2 classification based on stipule shape and flower color, the fact of linkage between the Aa and Yy factors is well established.

GRADUS ROGUE × MUMMY

The Gradus rogues used in the series of crosses with Mummy were all descended from a rogue plant, S15, selected in 1916 from a commercial planting of Gradus. Progeny of the same rogue, S15, when crossed with Gradus type behaved as typical rogues in inheritance (cultures 9.1270–9.1275, 9.1320, and 9.1321, Table II).

The F_1 plants (Table XII) of Gradus rogue × Mummy, and reciprocal had, with one exception, rogue stipules, and in this respect appeared similar to the pure rogues. As volunteer crossing occurred in the F_1 of Gradus rogue × Gradus, it might be expected to take place among the F_1 hybrids of rogue and Mummy. Since the F_1 plants in the latter cross exhibited most of the dominant Mendelian factors present in any of the strains growing in the experimental gardens in 1919, it would be difficult to detect volunteer hybrids in the F_2 generation (F_1 out-crosses). However, as the F_1 hybrids were very late in flowering, cross-pollination by insects, if not entirely absent was cut down to a minimum.

TABLE XII.—*Pedigrees of F_1 cultures of Gradus rogue × Mummy (P16) and reciprocal*

Culture.	Pedigree.	Mature plants.
9.720	(S15-2-5r x P16-10)—1 to 6	Rogues.
9.721	(S15-2-12r x P16-10)—1 to 9	Do.
9.722	(S15-2-3r x P16-10)—1 to 7	Do.
9.723	(S15-2-19r x P16-10)—1 to 6	Do.
9.725	(S15-1-6r x P16-10)—1 to 3	Do.
9.726	(S15-1-9r x P16-10)—1 to 8	Do.
9.727	(S15-3-1r x P16-10)—1 to 8	All rogues but No. 6, which resembled F_1 of P 16 x type.
9.728	(S15-3-4r x P16-10)—1 to 3	Rogues.
9.729	(S15-3-5r x P16-10)—1 to 8	Do.
9.730	(P16-12 x S15-4-5r)—1 to 11	Do.
	Total 69	

As some uncontrolled pollinations may have occurred, there is possibly an experimental error in the results which should be kept in mind when interpreting the F_2 data. On this account the crosses are being repeated, and it is planned to grow the F_1 generation under glass for protection. It is believed, however, that the data here presented are of sufficient interest to warrant publication at this time and that the errors from out-crossing will prove minimal.

RELATION OF THE A AND A FACTORS FOR FLOWER COLOR

The F_1 plants of the crosses between rogue and Mummy had colored (purple) flowers. In Table VIII is shown the percentage of colored-flowered (AA + Aa), segregates to white-flowered (aa), segregates in the F_2 generation of rogue × Mummy, and reciprocal. The observed percentages, 76.79 per cent AA and Aa plants and 23.21 per cent aa segregates conform to expectation.

RELATION OF A AND C, FACTORS FOR FLOWER COLOR

Gradus rogue × Mummy in the F_2 generation gave approximately the expected 56.25 per cent purples (with factors A, B, and C); 18.75 per cent pinks (with factors A and B); and 25.00 per cent whites (with factors aa), the observed percentages being 56.91 per cent, 19.88 per cent and 23.21 per cent, respectively, the deviations being well within the limits of error due to random sampling (Table IX).

Although the dovetailing of observation and theory is good for the three classes of color segregates as a whole, there is in the F_2 in both the case of Mummy × Gradus and Mummy × Gradus rogue, a deficiency of white segregates. The deficiency of aa plants can be explained by differences in disease resistance as well as by variations in time of maturing between colored and noncolored F_2 segregates. On the whole, plants with colored flowers are more resistant to disease than are the white flowered forms, so that proportionately more of the latter would be lost before they reached maturity. For unavoidable reasons the F_2 data were collected late in the season after a number of the plants had matured. Plants whose stipules were too dried to measure were not classified as to flower color but their occurrence was noted. Tschermak (*12*), Hoshino (7), and others have noted a correlation between the presence of the A factor and the habit of late flowering. In all probability the same correlation existed in these cultures. On this account, relatively more of the white segregates than of the colored ones were too mature to be used in this study at the time the stipule measurements were made.

RELATION OF N AND N FACTORS FOR NORMAL AND FASCIATED STEM, RESPECTIVELY

The 69 F_1 plants of Gradus rogue × Mummy had normal stems. No F_2 counts were made of the normal and fasciated plants, but apparently there occurred the expected Mendelian segregation.

RELATION OF Xx′ AND Yy FACTORS FOR STIPULE SHAPE

In 1919, 69 F_1 hybrids (Table XII) were grown from the seed of 13 pollinations made the preceding year. The young plants resembled Mummy in regard to stipule shape and were uniform in appearance.

With the exception of one plant (9.727–6), the hybrids were all classified as rogues at maturity. Plate 3, B, is of the upper part of a branch of a mature F_1 plant from Mummy × Gradus rogue.

The exceptional plant (9.727–6) resembled the F_1 of Mummy × Gradus, although 7 other plants from the same cross had rogue stipules. The atypical F_1 hybrid (9.727–6) produced 61 F_2 plants in 1920 (Culture No. 0.1084). At the time the notes were taken 14 individuals were too dried to classify. Of the 47 remaining plants, 23 were grouped as intermediates, with stipules intermediate in shape between those of Mummy and those of Gradus, and 24 were classed as Graduslike types. Apparently there were no rogues among the F_2 offspring.

Besides the F_2 family (0.1084) derived from the atypical F_1 plant, five other F_2 cultures were classified by inspection as rogues and nonrogues, the latter being subdivided into broads and intermediates (Tables XIII and XIV). The number of individuals in each of the three categories is shown in Table XIV. The ratio of rogues to nonrogues indicates a single factor difference between the two parents as being primarily responsible for the difference in stipule shape. The observed ratio of 77.12 per cent rogues to 22.88 per cent nonrogues agrees with that theoretically expected being within the error due to the limitations of random sampling.

On the assumption of a single factor pair, X and x′, primarily responsible for a difference in mean stipule ratio of 2.05 in Mummy and as compared with 2.35 in Gradus rogue, one would expect the F_2 to segregate only into rogues and Mummylike plants. Actually three classes based on stipule shape were distinguished, namely, rogues, intermediates (Mummylike), and broads (Table XIV), the latter having stipules comparable to those of Gradus.

TABLE XIII.—*Classification into rogues and nonrogues of five F_2 families from the cross Gradus rogue × Mummy*

Parent.	Culture No.	Rogues.	Nonrogues.		Unclassified.	Total.
			Intermediate.	Broad.		
1919:	1920:					
9.726–5.....	0.1076.....	79	19	3	8	109
9.726–6.....	0.1077.....	68	15	2	1	86
9.726–7.....	0.1078.....	8	4	2	2	16
9.726–8.....	0.1079.....	11	4	0	4	19
9.727–8.....	0.1085.....	16	4	1	6	27
		182	46	8	21	257

TABLE XIV.—*Relation of Xx′ and Yy in F_2 generation of cross Mummy×Gradus rogue (x′x′YY × XXyy), data from Table XIII.*

	XY or Xy rogues.	x′Y or x′y nonrogues.		Total.
		Intermediate.	Broads.	
Observed, 12:3:1 ratio	182	46	8	236
Percentage found	77.12	19.49	3.39	.....
Percentage expected	75.00	18.75	6.25	.....
Difference (D)	2.12	.74	2.86	.....
χ^2=2.8587				
P=.2231				
Observed, 3:1 ratio	182	54	.....	236
Percentage found	77.12	22.88	.....	.....
Percentage expected	75.00	25.00	.....	.....
Difference (D)	2.12	2.12	.....	.....
Standard error (S. E.)	1.89	.....	.....	.....
D/S. E.	1.12	.....	.....	.....

The presence of the broads in the F_2 generation of the cross Gradus rogue with Mummy is accounted for by assuming the presence of two factors y and x for stipule width in Gradus, factor y being allelomorphic to Y in Mummy and factor x allelomorphic to x′ found in Mummy. Ordinarily neither x nor x′ has any visible effect on stipule shape in either variety. Occasionally the factor x in Gradus mutates to X, which has for its somatic expression a mean stipule ratio of about 2.35. Factor X interacts with its allelomorph x as found in Gradus and is dominant to x′ of Mummy. It also masks either member of the factor pair Yy. Any F_2 segregate that is heterozygous or homozygous for the factor X has rogue stipules, irrespective of whether Y or y is present. An individual in which X is absent has intermediate or broad stipules, depending on the presence or absence, respectively, of the factor Y. In the F_2 generation the plants segregate into approximately 12 rogues; 3 intermediates; 1 broad, according to the following scheme:

P_1 ..... Gradus rogue, XXvy×Mummy, XX x′ x′
F_1 ..... Rogue, Xx′ Yy

F_2	Rogues:			Intermediates:		Broads:
	Xy	Xy		x′Y		x′y
Percentage	56.25	18.75		18.75		6.25
Ratio	12		:	3	:	1

	Rogues (X):		Non-rogues (X′):
Percentage	75		25
Ratio	3	:	1

	Rogues and Intermediates:		Broads:
Percentage	93.75		6.25
Ratio	15	:	1

Returning again to the results tabulated in Table XIV, the observed number in any one category of plants is expressed in percentages of the total number of plants classified. For the rogues, intermediates, and types the percentages are 77.12, 19.49, and 3.39, respectively, compared with the theoretically expected 75.00, 18.75, and 6.25 for the respective classes, the deviations of the observed percentages from the calculated are not statistically significant (P having a value of 0.2331). The greatest deviation is caused by the exceptionally low number of board-stipuled plants found. The deficiency in the broad class, however,

may be partially explained by assuming a differential mortality rate among the F_2 segregates as explained in the paragraph dealing with the relation of the Aa and Cc factors in the F_2 generation of Gradus rogue × Mummy.

The occurrence of the early flowering segregates offers a partial explanation of the deviation in the number of broad-stipuled plants observed from that theoretically expected, as there is in all likelihood a correlation in Gradus and Mummy between the shape of the stipules and time of maturing. As has been shown, the Graduslike character of the broad-stipuled F_2 plants was inherited through the rogue parent, which also carried the factors for early flowering. The results with Gradus × Mummy show that linkage exists in Gradus between the factors y for stipule shape and a for flower color. Hoshino (7) has shown the factor a to be linked with a factor for early flowering. Necessarily linkage exists in some degree between the factor y for stipule shape and the factor for early flowering. One is, therefore, justified in assuming that many of the white, broad-stipuled segregates would be early in maturing and the correlation would account for a part of the deviation of the observed number of broad F_2 types from the number theoretically expected.

STATISTICAL STUDY OF THE F_2 GENERATION OF THE CROSSES GRADUS ROGUE AND MUMMY

In 1920 were grown 51 F_2 families of the cross Gradus rogue with Mummy. The average ratio of stipule width to length in the mature F_2 plants was calculated in the same manner as for the P_1 generation. The F_2 segregates were also classified according to flower color but not described as broads, intermediates, or rogues.

RELATION OF COLOR FACTORS A AND a TO STIPULATE SHAPE IN TWO ATYPICAL FAMILIES

With the exception of the plant already mentioned,[5] all of the F_1 hybrids were rogues and appeared similar in regard to stipule shape. Although somatically alike the F_2 frequency distributions of the progeny of two apparently normal F_1 rogues are different from those of the remaining 49 F_2 families in both their range of variation and mean stipule ratio (Table XV). The means of the two families are 1.999 ± 0.9106 and 1.905 ± 0.0126 and for the two together the mean is 1.998 ± 0.0082 as compared with 2.333 ± 0.0050 for the other 49 F_2 cultures (Table XVI).

Because of their similarity both in frequency distribution and in the value of the mean stipule ratios, the two anomalous cultures have been lumped together for a statistical study of the color segregates. The means of the three color classes are: For the whites, 1.899 ± 0.0159; for the pinks, 2.046 ± 0.0167; for the purples, 2.038 ± 0.0095; and for colored plants as a whole, 2.027 ± 0.0085. The difference in means of the AA and aa as compared with aa plants is 6.7 times the probable error. The frequency distribution of the a plants resembles more that of Gradus than it does that of Mummy or Gradus rogue. The frequency distribution of the A population resembles that of Mummy rather than that of either Gradus or Gradus rogue. The difference in the mean stipule ratios of the two color groups, as well as the difference in their ranges

[5] The two exceptional F_2 families were cultures numbered 0.1059 and 0.1093 (Table XV), the progeny of F_1 plants 9.722–1 and 9.729–5, respectively (Table XII).

TABLE XV.—*Gradus rogue×Mummy, and reciprocal. Frequency distributions of ratio of* $\left(\frac{\text{length of stipule}}{\text{width of stipule}}\right)$ *of* F_2 *families*

Culture No.	1.55	1.65	1.75	1.85	1.95	2.05	2.15	2.25	2.35	2.45	2.55	2.65	2.75	2.85	2.95	3.05	Total.	M.	Coefficient variation.
0.1045	.....	1	0	1	4	4	3	1	5	7	2	3	2	2	.....	.....	35	2.325±0.0329	12.43±0.9848
0.1046	.....	1	1	1	0	0	3	4	3	6	3	1	.....	.....	.....	.....	23	2.245± .0374	11.85±1.1949
0.1047	.....	.....	.....	.....	1	1	0	0	3	3	1	1	0	0	0	1	11	2.359± .0390	8.14±1.6217
0.1048	.....	.....	1	1	1	3	4	4	7	7	11	12	3	3	1	1	59	2.466± .0231	10.75± .6751
0.1049	.....	.....	1	0	2	5	4	5	2	7	6	8	3	0	0	1	44	2.396± .0850	11.48± .0850
0.1050	.....	.....	1	1	4	1	2	5	9	9	2	3	2	1	.....	.....	40	2.340± .0264	10.59± .8075
0.1051	.....	.....	1	5	2	6	6	5	10	8	4	3	2	1	.....	.....	53	2.283± .0245	11.56± .7674
0.1052	.....	.....	.....	.....	2	2	0	1	1	4	1	2	1	.....	.....	.....	14	2.358± .0467	10.98±1.4190
0.1053	.....	.....	1	1	2	1	0	1	0	1	.....	.....	.....	.....	.....	.....	7	2.036± .0568	10.95±1.9973
0.1054	.....	1	1	3	4	3	3	5	6	5	11	9	2	4	3	.....	60	2.410± .0209	9.95±1.8113
0.1055	.....	.....	.....	1	2	3	3	4	4	5	4	1	2	.....	.....	.....	29	2.326± .0295	10.14± .9072
0.1056	.....	1	1	5	3	7	9	6	6	17	6	8	1	2	.....	.....	72	2.312± .0215	11.72± .6580
0.1057	.....	.....	1	1	1	9	5	1	2	5	1	0	2	.....	.....	.....	28	2.215± .0312	11.06±1.0033
0.1058	.....	.....	.....	.....	.....	2	0	2	2	2	1	1	.....	.....	.....	.....	10	2.340± .0397	7.94±1.2142
0.1059[a]	.....	1	3	9	23	24	6	3	2	.....	.....	.....	.....	.....	.....	.....	71	1.999± .0106	6.65± .3781
0.1060	.....	.....	.....	.....	.....	1	0	0	2	1	1	.....	.....	.....	.....	.....	5	2.350± .0494	6.97±1.5003
0.1061	.....	.....	2	0	3	3	1	0	1	.....	.....	.....	.....	.....	.....	.....	10	2.000± .0358	8.40±1.2758
0.1062	.....	.....	1	2	1	1	2	1	3	4	6	1	1	2	.....	.....	25	2.370± .0302	11.35±1.0966
0.1063	.....	.....	1	1	0	1	1	1	2	4	1	.....	.....	.....	.....	.....	12	2.160± .0522	12.41±1.7348
0.1064	.....	.....	.....	1	1	0	2	4	4	.....	.....	.....	.....	.....	.....	.....	12	2.208± .0302	7.02± .9684
0.1065	1	0	1	5	5	1	7	9	7	7	4	2	0	3	.....	.....	52	2.255± .0265	12.59± .8459
0.1066	.....	.....	1	1	3	1	1	3	4	3	3	3	1	.....	.....	.....	24	2.312± .0370	11.63±1.1492
0.1067	.....	.....	1	1	5	5	6	7	3	7	9	8	5	.....	.....	.....	57	2.357± .0256	12.17± .7798
0.1068	.....	1	1	2	3	2	4	1	2	4	10	7	1	2	.....	.....	40	2.357± .0322	12.81± .9817
0.1069	.....	.....	2	3	4	6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	15	2.215± .0313	12.59±1.0165
0.1070	.....	.....	.....	1	1	0	2	2	1	1	.....	.....	.....	.....	.....	.....	8	2.175± .0436	8.41±1.4282
0.1071	.....	.....	.....	2	0	2	3	1	4	0	4	1	2	.....	.....	.....	19	2.328± .0414	11.51±1.2760
0.1072	.....	.....	.....	.....	.....	.....	1	2	2	4	0	0	1	.....	.....	.....	10	2.390± .0334	6.65± .9998
0.1073	.....	.....	.....	.....	1	0	0	0	2	2	.....	.....	.....	.....	.....	.....	5	2.310± .0552	7.92±1.6998
0.1074	.....	.....	1	2	3	6	2	6	2	12	4	2	1	.....	.....	.....	41	2.309± .0248	10.22± .7691
0.1075	.....	.....	.....	.....	.....	4	0	1	1	4	2	0	2	.....	.....	.....	14	2.372± .0386	9.02±1.1563
0.1080	.....	.....	.....	1	0	0	2	1	4	2	0	1	0	1	1	.....	13	2.397± .0525	11.72±1.5715
0.1081	.....	.....	.....	.....	.....	.....	.....	2	3	1	3	2	1	0	2	.....	14	2.543± .0398	8.69±1.1160
0.1082	.....	.....	.....	.....	3	4	1	2	4	11	5	8	2	2	.....	.....	42	2.434± .0251	9.90± .7356
0.1083	.....	.....	.....	1	1	2	1	2	6	8	7	7	3	1	0	1	40	2.470± .0254	9.63± .7329
0.1086	.....	.....	.....	1	0	2	0	1	1	1	1	2	.....	.....	.....	.....	9	2.317± .0607	11.65±1.8772
0.1087	.....	.....	2	0	0	2	3	1	3	1	1	3	2	1	.....	.....	19	2.350± .0479	13.19±1.4682
0.1088	.....	.....	.....	.....	.....	3	0	0	4	4	2	3	1	1	1	.....	19	2.472± .0385	10.07±1.1050
0.1089	.....	.....	2	2	6	2	5	6	7	11	10	3	3	2	1	.....	60	2.350± .0245	11.91±1.4496
0.1090	.....	.....	.....	3	5	1	1	4	6	5	6	2	0	1	.....	.....	34	2.303± .0304	11.41± .9453
0.1091	.....	.....	1	0	2	1	2	1	5	1	1	1	.....	.....	.....	.....	15	2.250± .0466	10.35±1.2893
0.1092	.....	.....	.....	1	0	1	1	0	1	1	.....	.....	.....	.....	.....	.....	5	2.170± .0642	9.81±2.1125

0.1093[a]	.....	.....	2	4	5	18	21	6	0	2	1	.....	.....	.....	.....	.....	.....	59	1.905 ± .0139	7.72 ± .4822
0.1094	.....	4	2	41	15	5	3	0	4	2	4	4	1	.....	.....	.....	.....	53	2.094 ± .0224	11.55 ± .7667
0.1095	.....	1	0	3	4	6	4	13	12	18	5	3	1	1	.....	.....	.....	71	2.306 ± .0173	9.36 ± .5338
0.1096	.....	.....	.....	.....	1	3	0	0	0	2	.....	.....	.....	.....	.....	.....	.....	4	2.250 ± .0951	12.53 ± .9347
0.1097	.....	.....	2	.....	7	6	8	6	14	16	19	9	8	7	3	.....	.....	119	2.385 ± .0181	12.88 ± .5725
0.1098	.....	.....	.....	.....	1	2	1	0	1	0	0	1	.....	.....	.....	.....	.....	6	2.117 ± .0738	12.65 ± .5023
0.1099	.....	.....	.....	.....	.....	.....	.....	1	1	4	2	2	3	0	1	.....	.....	14	2.471 ± .0326	7.32 ± 1.6792
0.1100	.....	.....	.....	.....	.....	.....	1	0	0	0	1	0	1	1	.....	.....	.....	4	2.475 ± .0904	10.82 ± 2.6185
0.1101	.....	.....	.....	1	0	0	1	2	0	2	0	1	0	0	1	.....	.....	8	2.275 ± .0741	13.67 ± 2.3478
Total	.....	1	11	37	86	145	167	122	136	187	231	169	131	58	39	12	4	1,534	2.307 ± .0048	12.48 ± .1542

[a] Atypical cultures 0.1059 and 0.1093.

of variation, indicates linkage in the two parents between the factors Yy for stipule width and the Aa factors for flower color. Recombinations in the F_2 of cross-over and noncross-over gametes would account for differences in the range of variation and in the means between the F_2 A and a segregates and the A and a parents.

However, as the combined frequency distributions of the two crosses of Mummy with rogue cultures under discussion (0.1059 and 0.1093) show considerable variation from the F_2 frequency distribution of Gradus as type × Mummy, especially in the position of the respective modes and means, it is unlikely that the factor allelomorphic to x′ in the F_1 hybrid is the x of Gradus type; it is apparently a mutation of X to some other factor for stipule shape, the mutation being analogous to the genetic change occurring when intergrading intermediates are produced from Early Giant types (*1*). In this case the intermediates are of the sort producing few or no rogues among their progeny.

Although the ranges of variation of the two atypical F_2 progenies (0.1059 and 0.1093) overlaps the range of Gradus rogue, no rogues were present in the cultures judging from the character of the F_3 generation. Six F_2 plants of culture 0.1093 from classes 2.05, 2.15, and 2.45 of the variation curve gave only broad and intermediate F_3 segregates. It would appear that the rogue factors which entered the F_1 zygote failed to function as rogue factors at gametogenesis, having mutated to something different from X.

RELATION OF COLOR FACTORS A AND C TO STIPULE SHAPE IN THE TYPICAL F_2 FAMILIES OF THE CROSSES OF MUMMY WITH GRADUS ROGUE

The stipules of 1,404 plants, progeny of the remaining F_2 families (omitting cultures 0.1059 and 0.1093), were measured. The plants represented 49 families, of which the frequency distributions and the derived statistical constants are given in Table XV.

Of the 1,404 individuals, 356 were white (of constitution aa); 305 pink (with A and B factors); and 873 were purple (with A, B, and C factors). The mean stipule ratio of the white segregates was found to be 2.258 ± 0.0110 as compared with 2.336 ± 0.0110 for the pinks and 2.325 ± 0.0063 for the purples (Table VII).

As no essential difference exists between the means of the pink and purple F_2 plants, and as they each contain the A factor, the two color populations may be combined for comparison with the aa segregates. The mean of the AA and Aa plants is 2.335 ± 0.0056 as contrasted with 2.258 ± 0.0110 for the aa segregates, a difference in the means of over 6 times the probable error of the difference.

For a comparison inter se of the frequency distributions of the AA and Aa, and aa segregates and of the F_2 as a whole the number of variates in each class was expressed in percentages of the total number of variates in that particular category of plants. Figure 3 shows the curves obtained by plotting the percentage of variates in each class for the AA and Aa, and aa segregates separately, and for all the F_2 plants irrespective of the color factors present. The graphs for Gradus, Gradus rogue, and Mummy are shown in figure 2, for comparison with the graphs of the F_2 generation.

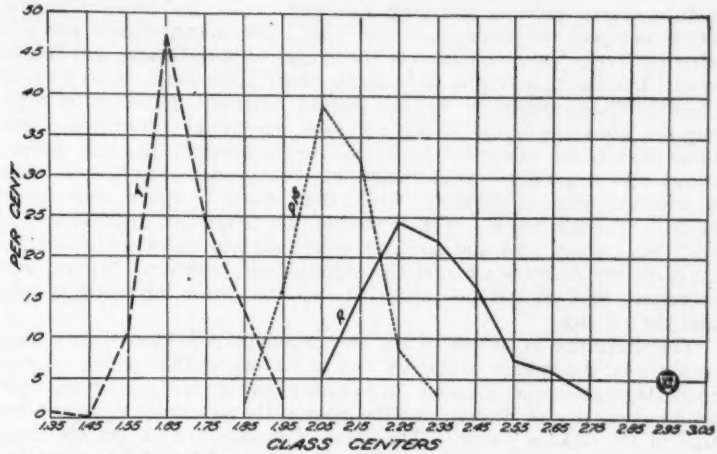


FIG. 2.—Graphs obtained by plotting the frequency distributions, expressed in percentages, of ratio of $\frac{\text{length of stipule}}{\text{width of stipule}}$ for Gradus (T), Mummy (M), and Gradus rogue (R), based on data in Table VIII.

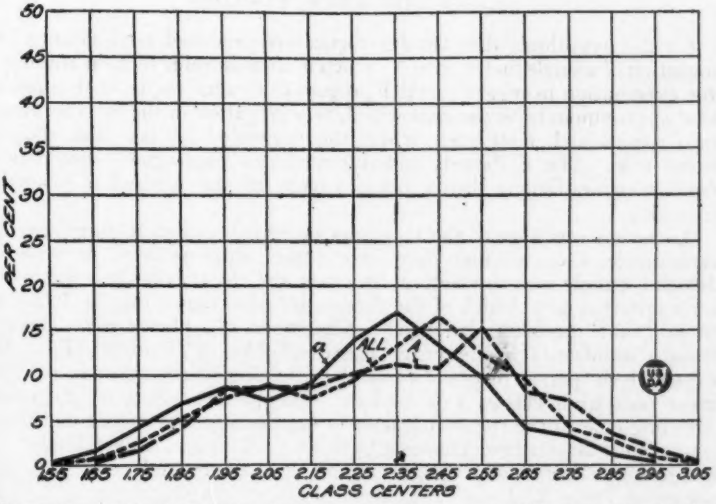


FIG. 3.—Graphs obtained by plotting the frequency distributions, expressed in percentages, of ratio of $\frac{\text{length of stipule}}{\text{width of stipule}}$ for all the F_2 variates of Mummy × Gradus rogue and for the AA and Aa segregates (A) and aa segregates (a) taken separately, based on data in Table VIII.

The majority of variates in a population of Gradus types have a mean stipule ratio between 1.50 and 1.79 and none have been observed with a stipule ratio above 1.99. For Gradus rogue no plants have been found with a stipule ratio of less than 2.00 and by far the greatest number occur between the limits 2.10 and 2.49. The mean stipule range of Mummy extends over portions of the ranges of both Gradus and Gradus rogue, but the majority of individuals occur in classes 1.95 to 2.15, inclusive. Consequently, if linkage existed between the factor for rogue stipules and the a factor for white flowers, one would expect proportionately more of the aa variates to lie close to the mean of the rogue parent, 2.339, and a greater concentration of the variates with the A factor around the mean of Mummy, 2.088, than would be found close to the mean of Gradus rogue. The modes of the frequency distributions of both the colored (AA and Aa) and white (aa) segregates are actually at 2.45, or very near the mean of the rogue parent, however. It is evident, therefore, that no linkage exists between the factor for rogue stipules and the a factor.

The difference in means of the two populations is caused in a large measure by the linkage of the AY and ay factors, so that among the nonrogue segregates the AA and Aa variates tend to have a stipule ratio approaching that of Mummy and the mean of the aa segregates approaches that of the Gradus parent. The effect of linkage is to lower the mean of the aa plants and to increase the mean of the AA and Aa segregates. For this reason more aa segregates than AA and Aa segregates are found in classes 1.55 to 1.95, inclusive. Linkage of the two factors also accounts for the tendency to bimodality that both curves exhibit. (Fig. 3.)

GRADUS ROGUE X MUMMY, AND RECIPROCAL IN THE F_3 GENERATION; CLASSIFICATION OF F_3 FAMILIES

On the hypothesis that Gradus rogues are produced from Gradus by mutation of a single factor, x to X, which is allelomorphic to x′ in Mummy, the expectation in regard to the F_2 rogues of Gradus rogue × Mummy is that approximately 66 per cent will show segregation in the F_3 generation into rogues and nonrogues, while the remaining 33 per cent should breed true. The F_2 broads and intermediates (nonrogues) should not contain rogues among their progeny except as the product of primary mutation.

No record was kept of the F_2 plants saved for seed as to their general appearance, i. e., whether they were rogues, intermediates, or broads. Instead, stipule measurements were made and the stipule ratio was used for a criterion as to which of the three categories, rogue, intermediate, or broad, that the plant belonged. Altogether 184 plants were chosen from a number of F_2 frequency classes (Tables XVI to XVIII). As expected, the parent plants of families containing no rogues came for the most part from classes 1.65 to 2.25. The parent plants of progenies breeding true to the rogue character or segregating into rogues and nonrogues were largely from classes 2.35 to 2.95. This and a classification of seregating F_3 families of Gradus rogue × Mummy and reciprocal are shown in Table XVI.

TABLE XVI.—*Families in which no rogues appeared. Generic constitution of F_2 parents was* x′x′YY, x′x′Yy, *or* x′x′yy

Culture No.	Stipule ratio of F_1 parent.	Broads.	Broads and intermediates.	Intermediates.	Total.
0.279	.....	9	.....	.....	9
0.281	1.65	29	.....	.....	29
0.283	.....	.....	31	.....	31
0.285	.....	.....	.....	13	.....
0.289	.....	.....	16	.....	16
0.291	.....	27	.....	.....	27
0.293	.....	.....	25	.....	25
0.294	.....	.....	16	.....	16
0.296	.....	.....	44	.....	44
0.297	.....	.....	35	.....	35
0.298	1.75	.....	21	.....	21
0.299	.....	18	.....	.....	18
0.300	.....	.....	24	.....	24
0.301	.....	.....	61	.....	61
0.302	.....	.....	49	.....	49
0.303	.....	.....	33	.....	33
0.304	.....	.....	.....	38	38
0.305	.....	.....	.....	37	37
0.306	.....	.....	35	.....	35
0.308	.....	.....	12	.....	12
0.309	.....	.....	37	.....	37
0.310	.....	13	.....	.....	13
0.311	.....	46	.....	.....	46
0.314	.....	21	.....	.....	21
0.315	.....	.....	23	.....	23
0.316	.....	.....	73	.....	73
0.317	1.85	.....	44	.....	44
0.318	.....	.....	21	.....	21
0.319	.....	.....	25	.....	25
0.321	.....	.....	38	.....	38
0.322	.....	.....	91	.....	91
0.323	.....	.....	62	.....	62
0.324	.....	.....	70	.....	70
0.325	.....	.....	20	.....	20
0.326	.....	.....	38	.....	38
0.327	.....	.....	74	.....	74
0.328	.....	.....	11	.....	11
0.330	.....	.....	47	.....	47
0.331	.....	.....	20	.....	20
0.332	.....	.....	22	.....	22
0.340	.....	.....	71	.....	71
0.341	2.05	58	.....	.....	58
0.344	.....	.....	30	.....	30
0.345	.....	.....	47	.....	47
0.347	.....	.....	25	.....	25
0.348	.....	.....	23	.....	23
0.428	.....	.....	75	.....	75
0.429	.....	.....	31	.....	31
0.432	2.25	.....	42	.....	42
0.433	.....	.....	110	.....	110
0.454	2.35	.....	99	.....	99
0.466	2.45	.....	42	.....	42

TABLE XVII.—*Families segregating into rogues and nonrogues. Genetic constitution of F_2 parents was $Xx'YY$, $Xx'Yy$, or $Xx'yy$*

Culture No.	Stipule ratio of F_3 parent.	Rogues.	Nonrogues.	Total.
0.307	1.85	31	11	42
0.329		18	8	26
0.333		?	?	70
0.418		?	?	38
0.420		18	2	20
0.421	2.25	16	3	19
0.422		27	3	30
0.425		31	2	33
0.426		69	4	73
0.431		34	4	38
0.436		18	2	20
0.440		24	3	27
0.441		21	2	23
0.443		19	3	22
0.446		31	6	37
0.450		33	2	35
0.451	2.35	34	3	37
0.453		32	2	34
0.457		57	3	60
0.459		36	2	38
0.460		117	13	130
0.461		64	5	69
0.462		33	4	37
0.465		88	6	94
0.466		25	4	29
0.470		23	5	28
0.471		25	3	28
0.472		14	9	23
0.473		29	3	32
0.474		12	1	13
0.477		13	4	17
0.482	2.45	15	8	23
0.484		29	4	33
0.486		22	6	28
0.489		28	7	35
0.491		24	7	31
0.492		37	9	46
0.493		14	2	16
0.497		19	4	23
0.500		18	2	20
0.502		6	5	11
0.503		16	5	21
0.2419		66	9	75
0.2420		36	5	41
0.2423		28	14	42
0.2424		58	3	61
0.2427		46	3	49
0.2428		25	6	31
0.2429	2.55	26	3	29
0.2430		11	4	15
0.2434		51	2	53
0.2439		?	?	36
0.2441		?	?	11
0.2442		?	?	51
0.2443		6	3	9
0.2444		6	1	7
0.2447		25	2	27
0.2448		18	6	24
0.2449		57	3	60

TABLE XVII.—*Families segregating into rogues and nonrogues. Generic constitution of F_2 parents was Xx′YY, Xx′Yy, Xx′yy*—Continued

Culture No.	Stipule ratio of F_2 parent.	Rogues.	Nonrogues.	Total.
0.2451	.....	49	12	61
0.2452	.....	29	2	31
0.2458	.....	35	2	37
0.2462	2.65	10	2	12
0.2463	.....	15	3	18
0.2464	.....	20	1	21
0.2466	.....	6	2	8
0.2467	.....	9	2	11
0.2468	.....	19	4	23
0.2473	.....	5	4	9
0.2475	.....	34	3	37
0.2476	2.75	31	5	36
0.2477	.....	40	8	48
0.2484	2.85	19	7	26
0.2489	.....	18	1	19
0.2490	2.95	39	5	44
0.2491	.....	25	2	27
Total	.....	2,097	308	.....
0.423 [a]	.....	7	57	64
0.424 [a]	2.25	4	19	23
0.430 [a]	.....	7	23	30
0.439 [a]	2.35	4	33	37
0.455 [a]	.....	3	67	70
0.469 [a]	2.45	4	54	58
0.574 [a]	.....	6	8	14
0.2445 [a]	2.65	13	19	32
0.2470 [a]	.....	12	17	29
0.2482 [a]	2.75	10	14	24
Total	.....	70	311	.....
Grand total	.....	2,167	619	.....

[a] Cultures containing an excess of nonrogues over rogues. The nonrogues were comparable to the intergrading intermediates described by Bateson and Pellew (*1*).

Of the 184 F_2 plants, 52 had progenies containing (1) only broads, (2) both intermediates and broads, or (3) intermediates only; i. e., they were recessive for the X factor but may have contained the Y or y factor. There were 86 progenies which segregated into rogues and nonrogues; while the progenies of 46 plants were all rogues (Tables XVI to XVIII. The observed number of XX : Xx′ F_2 segregates is very close to that theoretically expected (Table XIX).

Inspection showed segregating families to be of two kinds: First, those with an excess of rogues over nonrogues, and second, those with an excess of nonrogues over rogues. In the latter case the nonrogues were often comparable in appearance to the intergrading intermediates described by Bateson and Pellew (*2*). Except in cultures containing a very few plants, one would not expect such an excess of recessives as was found in these families.

TABLE XVIII.—*Families in which only rogues appeared. Genetic constitution of F_2 parents was XXYY, XXYy, or XXyy*

Culture No.	Stipule ratio of F_2 parent.	Rogues.	Culture No.	Stipule ratio of F_2 parent.	Rogues.
0.419		55	0.2425		42
0.427	2.25	49	0.2426	2.55	38
0.434		50	0.2435		32
0.442		25	0.2436		61
0.444	2.35	23	0.2437		44
0.445		52	0.2440		41
0.456		30	0.2418		34
0.463		18	0.2446		36
0.476		16	0.2450		16
0.478		29	0.2454		27
0.480		15	0.2455		20
0.481		21	0.2456		33
0.483		18	0.2457	2.65	28
0.485		26	0.2459		31
0.487	2.45	24	0.2460		19
0.488		17	0.2465		51
0.495		20	0.2469		17
0.496		13	0.2471		23
0.498		42	0.2472		18
0.499		39	0.2479	2.75	34
0.502		14	0.2483		44
0.2421		34	0.2486	2.85	15
0.2422		35	0.2487		17

TABLE XIX.—*Ratio of XX : Xx′ plants among 132 F_2 segregates of Gradus rogue × Mummy, as determined from analysis of the F_3 generation, based on data in Table XVII. The expectation is two segregating and one nonsegregating F_3 families*

	Nonsegregating.[a] XX.[b]	Segregating.[a] Xx′[b]	Total.
Observed	46	86	132
Calculated	44	88	132
Difference (D)	2	2	.....
Standard error (S. E.)	3.5	3.5	.....
D/S. E.	.5	.5	.....

[a] Character of F_3 family. [b] Constitution of F_2 parent.

The total number of rogues (2,097) in the cultures containing few nonrogues is obviously far in excess of the total number of nonrogues (308) that would be expected if normal Mendelian segregation occurred in the F_2 plants heterozygous for factors X and x′ (Tables XVI, XVII, XVIII). In the families producing many nonrogues and few rogues the situation is reversed, the cultures containing in all 70 rogues and 311 nonrogues, plainly not in accord with the theoretical, monohybrid 3 : 1 ratio shown in the same tables.

Disregarding the two types of F_3 families and considering all the segregating families as a whole, the observed numbers are: 2,167 rogues (XX and Xx′) and 619 nonrogues (x′x′). The numbers actually found in the two groups show an excess of 78 individuals in the rogue class, a deviation of nearly 4 times the probable error of the difference between the observed and calculated number of variates (Table XX).

TABLE XX.—*Ratio of rogue (XX and Xx') segregates to nonrogue (x'x') segregates in all F_3 cultures segregating into rogues and nonrogues, based on data in Table XVII*

	XX and Xx'.	x'x'.	Total.
Observed	2167	619	2786
Calculated	2089.5	696.5	2786
Difference (D)	77.5	77.5	.....
Standard error (S. E.)	22.85	.....	.....
D/S. E	3.4	.....	.....

STATISTICAL STUDY OF STIPULE SHAPE IN THE F_3 FAMILIES OF GRADUS ROGUE X MUMMY, AND RECIPROCAL

Besides classifying the F_3 cultures on the basis of stipule character a number of families were chosen for a statistical study of the stipule shape. A record was kept of whether the families consisted of nonrogues only, rogues, or of both rogues and nonrogues. Table XX shows the frequency distributions and means of the cultures measured. In general the mean stipule ratio of each family tends toward the mean of the category to which the F_2 parent belonged (Table XXI). Thus the means of the x'x' families tend to approach the mean of x'x' parent (Mummy) while the Xx' and XX families have means close to that of the XX parent (Gradus rogue). This tendency in the means of the three types of families is greater if the frequency distributions of the several families belonging to each group be combined. In Table XXII are shown the total frequency distributions from Table XXI of 28 segregating families, of 8 families breeding true to the rogue character, and of 15 families in which no rogues occurred. The mean of the rogue families is 2.503 ± 0.0093 as compared with 2.339 ± 0.0096 for Gradus rogue. The increased narrowness of the stipules of the rogue segregates over that of the rogue parent may be due to the accumulative effect of factors modifying stipule shape such as the Y and n factors responsible for stipule shape and stem fasciation, respectively, in Mummy. The mean of the segregating cultures is 2.259 ± 0.0062 or very near the mean, 2.333 ± 0.0050, of all the F_2 cultures of Gradus rogue × Mummy. For the families in which no rogues appeared the mean is 1.899 ± 0.0053 or intermediate between the means of Gradus type and Mummy, 1.693 ± 0.0107 and 2.088 ± 0.0050, respectively. The mean of the x'x' families is especially significant as indicating a segregation into Graduslike plants and Mummylike plants among the F_3 plants lacking the X factor. Such a segregation is to be expected in the progeny of F_2 plants of the composition x'x'Yy, and from the effect of lumping together into one frequency distribution the F_3 families with formulae x'x'YY and x'x'yy.

The evidence from the statistical study of stipule shape in the F_3 families points to the homozygous nature of the broad or intermediate F_2 segregates in regard to the x' factor. In addition the occurrence of F_2 rogues homozygous and heterozygous for the X factor is also demonstrated by measurement of the stipules of their F_3 progenies.

TABLE XXI.—*Frequency distributions and statistical constants of ratio of* $\left\{\frac{\text{length of stipule}}{\text{width of stipule}}\right\}$ *for the F_3 families of Gradus rogue×Mummy. The rogue families (indicated by R), the nonrogue families (indicated by N), and the families segregating into rogues and nonrogues (indicated by S), are marked in the extreme right-hand column.*

Culture No.	Characters of F_2 parent.	1.45	1.55	1.65	1.75	1.85	1.95	2.05	2.15	2.25	2.35	2.45	2.55	2.65	2.75	2.85	2.95	3.05	3.15	3.25	3.35	Total.	M.	Coefficient variation.	Classification of F_3 family.
1.279	1.65			1	1	4	2	1														9	1.961±0.0245	5.60±0.8931	N
1.281			2	6	6	10	5															29	1.792± .0136	6.08± .5332	N
1.283				1	11	9	7	3														31	1.850± .0126	5.62± .4829	N
1.285	1.75				3	4	6															13	1.873± .0072	2.08± .2649	N
1.289					2	3	4	7														16	1.950± .0178	5.43± .6493	N
1.293				3	0	3	11	4	4													25	1.950± .0188	7.18± .6884	N
1.294					2	3	2	3	4	1												15	2.056± .0315	9.09±1.0817	N
1.301		1	2	21	16	11	7	1	0	1												60	1.759± .0117	7.65± .5069	N
1.304				2	5	15	4	3	1	2	0	1	2									35	1.941± .0247	11.17± .9036	N
1.306				1	1	2	6	13	7	2												32	2.050± .0137	5.65± .4778	N
1.307	1.85					5	6	4	7	5	3	7	2	3								42	2.231± .0247	10.67± .7941	S
1.309		1	1	0	0	0	11	12	7	2	1	2										37	2.050± .0184	8.09± .6393	S
1.314				1	7	7	6															21	1.864± .0035	1.28±1.3325	S
1.329					2	4	4	6	3	2	3	0	1	0	1							26	2.089± .0349	12.63±1.2000	S
1.308					2	3	2	0	3	2												12	1.991± .0348	8.99±1.2491	N
1.325				2	6	6	10	7	4													35	1.925± .0133	6.07± .4854	N
1.341				2	6	16	11	7	1													43	1.917± .0114	5.79± .4225	N
1.342	1.95				2	2	3	2	2	7	10	10	0	3	1	2						44	2.308± .0262	11.17± .8131	R
1.343								1	2	4	3	4	2									16	2.331± .0239	6.09± .7179	R
1.346				2	2	10	14	8	1	3	2	1	0	2	1							46	2.124± .0239	11.34± .8076	S
1.347					1	7	7	5	2	2	1											25	1.990± .0199	7.43± .7127	S
1.333	2.15				3	0	0	5	11	12	16	10	5	5	1	2						70	2.336± .0179	9.54± .5487	S
1.418					3	3	8	4	4	6	5	3	1									37	2.117± .0242	10.01± .7919	S
1.419								1	7	7	10	14	10	4	1	1						35	2.427± .0140	6.34± .4093	R
1.423	2.25				3	4	9	9	11	10	7	3	5	1	2							64	2.071± .0196	11.26± .6786	S
1.425					1	1	1	4	5	4	3	7	3	2	1	1						33	2.207± .0328	12.68± .0695	S
1.428						2	9	12	1	1												25	2.010± .0066	2.43± .2319	N
1.430					3	5	8	4	1	0	1	0	2	1								25	2.022± .0308	11.32±1.0930	S
1.445	2.35			1	6	6	9	9	1	3	1	4	2	0	2	0	0	1				45	2.132± .0297	13.88±1.0057	S
1.446				1	2	7	2	3	5	9	3	3	1	0	1							37	2.068± .0248	10.69± .8477	S
1.450					3	3	5	9	4	3	2	3	2	1								35	2.138± .0261	10.71± .8741	S
1.458						6	7	8	12	7	6	1	0	2								49	2.128± .0168	8.22± .5638	S
1.467	2.45			1	1	2	8	2	7	4	7	3	3									38	2.165± .0267	10.77± .8429	S
1.2437									1	5	9	2	6	10	3	5	2	1				44	2.606± .0220	8.32± .6023	R
1.2438							2	0	1	3	3	2	3	1	1							16	2.362± .0364	9.99±1.2041	S
1.2440	2.55						1	0	1	5	6	7	18	3	0	0	1					42	2.462± .0147	5.76± .4253	R
1.2441					1	1	3	1	1	3	0	3	1									14	2.164± .0440	11.27±1.4548	S
1.2442				2	3	9	3	5	7	8	10	2	1									50	2.126± .0294	14.58±1.0041	S

I.2449	2.65	...	...	...	1	0	2	4	4	2	7	8	9	9	3	1	...	...	...	...	...	50	2.418± .0231	10.04± .6812	S
I.2451		...	...	2	2	3	6	7	5	12	7	6	4	2	1	0	1	...	...	...	...	58	2.229± .0231	11.71± .7433	S
I.2452		...	...	...	...	1	1	2	1	3	4	4	4	2	2	1	...	...	...	...	...	25	2.394± .0337	10.41±1.0067	S
I.2454		...	...	...	...	...	...	...	4	2	3	7	6	1	2	0	2	...	...	...	...	27	2.469± .0259	8.10± .7483	R
I.2455		...	...	...	...	...	...	2	1	2	4	2	5	1	2	1	...	...	...	...	...	20	2.440± .0322	8.77± .9442	R
I.2456		...	...	...	...	...	...	...	...	2	2	5	9	5	4	3	2	0	2	...	...	34	2.562± .0270	9.13± .0191	R
I.2458		...	...	...	...	...	1	2	4	0	2	3	6	6	3	5	0	1	...	...	...	33	2.462± .0321	11.29± .9495	S
I.2470		...	...	...	2	0	2	1	0	3	1	5	3	4	0	1	...	...	...	...	...	22	2.359± .0451	13.31±1.3772	S
I.2472		...	...	...	...	...	...	...	1	0	3	0	1	3	4	2	5	2	...	...	...	21	2.727± .0348	9.05± .9442	R
I.2476		...	...	...	...	...	1	0	4	4	10	8	4	2	1	1	...	...	...	...	...	35	2.392± .0203	7.44± .6031	S
I.2477	2.75	...	...	...	...	...	...	...	3	3	1	4	6	6	6	6	2	4	3	0	2	46	2.709± .0303	11.25± .8010	S
I.2483	2.85	...	...	...	...	...	1	2	0	2	3	4	5	6	10	4	5	1	0	1	1	45	2.652± .0282	10.59± .7614	S
I.2490	2.95	...	...	...	1	0	0	5	2	3	2	2	2	6	5	4	4	1	0	0	1	38	2.556± .0392	13.26±1.0438	S
I.2440		...	...	1	1	3	1	7	8	10	2	1	...	...	...	...	...	...	...	...	...				
I.2441		...	...	2	3	9	3	5	7																
I.2442		...	...																						

TABLE XXII.—*The combined frequency distributions and derived statistical constants of ratio of* $\left\{\frac{\text{length of stipule}}{\text{width of stipule}}\right\}$ *for the rogue (R), segregating (S), and the nonrogue (N) families of the F_3 generation from cross Gradus rogue × Mummy based on data in Table XXI.*

Cultures. No.	Classification.	1.45	1.55	1.65	1.75	1.85	1.95	2.05	2.15	2.25	2.35	2.45	2.55	2.65	2.75	2.85	2.95	3.05	3.15	3.25	3.35	Total.	M.	s.	Coefficient variation.
8	R	...	...	...	...	...	1	4	17	37	40	41	57	27	16	12	12	3	1	...	...	258	2.503±.0093	0.2230±.0066	8.89±.2655
28	S	1	1	13	47	84	120	130	119	119	121	108	73	61	41	29	12	8	3	1	4	1,095	2.259±.0062	.306±.0044	13.54±.1911
15	N	1	4	37	64	87	97	64	27	9	1	...	...	...	...	...	...	...	...	...	...	391	1.899±.0053	.154±.0037	8.81±.2141
Total		2	5	50	111	171	218	198	163	155	162	149	130	88	57	41	24	11	5	1	4	1,745			

DISCUSSION OF RESULTS.

True-breeding hybrids have been found in other genera than Pisum, notably in *Oenothera*, and have been interpreted by Muller (*10*) as due to the action of balanced lethal factors. In self-fertilized Gradus plants there is no evidence of zygotic lethals since under proper growing conditions all the ovules in a pod develop. Although an occasional sterile rogue is met with, all ovules generally form viable seed. In over 60 instances crosses between Gradus and Gradus rogue gave an average of 4.5 seeds per pod out of a possible 7 or 9. About 4 seeds per pollination is the number generally obtained from artificial crosses of Gradus with other varieties. However, it has been customary to pollinate two or three stigmas with the pollen of one flower, the result being one well filled pod and one or two partially filled, as a consequence of deficient pollination.

A microscopical examination of the pollen of Gradus and Gradus rogue showed well-developed pollen grains in both forms. Their pollen is about 100 per cent perfect. There was no morphological evidence of defective pollen which might be interpreted as due to the action of gametic lethals.

It is, therefore, apparently out of the question to explain the anomalous behavior of the rogues in crosses with Gradus types as due to the presence of zygotic or gametic lethals.

As previously stated, the nonappearance of Gradus segregates in the F_2 generation of the cross Gradus with Gradus rogue is explained by Bateson and Pellew (*1*) as probably due to somatic segregation of the type and rogue "elements" in the F_1 hybrid. The same authors have not suggested, however, a mechanism by which somatic segregation is accomplished. Such segregation would occur if somatic nondisjunction took place early in the development of the F_1 plants, so that of the two daughter cells formed, one would receive both of the chromosomes derived from one member of the heterozygous chromosome pair present in the parent cell, and the other daughter cell would receive the two chromosomes derived from the second member of the heterozygous pair. As a result, both cells would retain the characteristic number of somatic chromosomes and at the same time be homozygous for either the type or rogue factors.

Besides assuming the phenomenon of somatic nondisjunction, the further assumption must be made that the daughter cells homozygous for the rogue factors increase more rapidly than cells containing the type factors, and consequently the F_1 plant becomes more roguelike as it matures. At sporogenesis only rogue tissue would take part in gamete formation; therefore type plants would not appear among the F_2 progeny.

Although somatic nondisjunction offers a hypothesis to account for somatic segregation it does not explain satisfactorily the failure of the type factors or "elements" to be present in the gametes of the F_1 hybrids. Certainly accepted instances of somatic nondisjunction are rare, the best examples being the gynandromorphs in *Drosophila* described by Morgan and Bridges (*9*) and attributed to the effect of the dropping out of the sex chromosome at an early division of the fertilized egg. The demonstration of the loss of a chromosome was possible from a knowledge of the factors linked with sex. As there are no factors known which are linked to the type allelomorphs of the rogue factors, the loss, by nondisjunction in the F_1 hybrid, of the chromosome carrying the type factor can not be detected. In addition, in the F_1 plants nondisjunctional

nuclear divisions would have to take place too regularly and too often to make the explanation seem reasonable.

As an alternative hypothesis, the idea of "mass" somatic mutation is advanced to account for the anomalous behavior of the rogues in heredity. Using the factor symbols here adopted the x factor of Gradus is assumed to mutate occasionally to the X factor of Gradus rogue. The factor pair xx when in a homozygous condition is relatively stable and mutation to X rarely takes place. However, the heterozygous Xx combination is very unstable and x mutates with great frequency to X, creating a homozygous and more stable condition of the germplasm in regard to the X factor. Mass somatic mutation of x to X occurs in the soma of the F_1 hybrid at an early stage in its development and would account for the prevailing absence of the x factor among the F_1 gametes. The same phenomenon would be true of Gradus plants in which the primary mutation of x to X took place. Such a mutation occurring early in the ontogeny of a Gradus type plant would produce an individual which would gradually become more roguelike as it matured. Or when the mutation takes place late in the development of the plant, the form of the individual would not necessarily be changed but the issue derived from the cell containing the X factor, would, if it entered into gamete formation, cause rogues to appear in the next generation.

The fact that occasionally an F_1 hybrid of Gradus × Gradus rogue is Graduslike at maturity rather than roguelike is interpreted as indicating that rare Gradus gametes are produced, as a consequence of mutation or otherwise, in which the X factor is replaced by an allelomorph comparable in stability to the x′ of Mummy.

It is probable that the rogues are an extreme manifestation of a series of mutations originating by changes in the x factor of Gradus. Thus the various sorts of intergrading intermediates described by Bateson and Pellew (1) may well represent various modifications of the factor x which may be designated as x″, x‴, etc. The xx″ or x″ x″ combinations produce intergrading intermediates in which mutation of either of x or x″ to X is more frequent than in Gradus type. The differences in the stability of the various modified x factors as exhibited in the rate of change of these factors to X, accounts for the existence of high and low rogue-producing strains of intermediates.

The difference in the proportion of rogues produced from the lower as compared with the upper nodes of intergrading intermediates, besides being due to a multiplication of the somatic cells in which a primary mutation of x to X has occurred, may conceivably be caused by an increase in the number of somatic mutations brought about by physiological changes in the protoplasm as the plant matures.

The mutation of x to X in Gradus, in the F_1 hybrid and in the intergrading intermediate is comparable to what Emerson (5) calls a recurring somatic mutation. The varieties in which rogues arise are of the sort earlier described by de Vries (13) as "ever-sporting," meaning that it is impossible to free the stock of the tendency to produce "sports" (mutations).

The results in the F_2 generation of crossing Gradus type with Mummy and Gradus rogue with Mummy have led to the assumption of a factor pair Yy in addition to the xx factors as partly responsible for stipule shape in Gradus and Gradus rogue. In Gradus there are at least two factor pairs xx and yy determining the ratio of width to length of stipule.

The y factor in Gradus is linked with the a factor for white flowers; its allelomorph Y is linked with the A factor for colored flowers in Mummy. The proof of the presence of two factors for stipule shape in Gradus and Gradus rogue lies in the fact that no linkage exists between the X factor (the mutated form of the x factor of Gradus) in Gradus rogue and the a factor for white flowers as determined from an analysis of the F_2 generation of the cross Gradus rogue with Mummy.

The allelomorph, in Mummy, of factor x is probably not identical with x but is very similar in its somatic expression. At least in combination with y of Gradus (the x′x′yy segregates from the F_2 generation of Rogue × Mummy), the x′x′yy plants have stipules very nearly like Gradus type. The factor allelomorphic to x and found in Mummy is called x′ to distinguish it from the x factor of Gradus. Additional evidence of a difference between x and x′ is the fact that x′ has never been known to mutate to X when x′ is present in the homozygous state. The factor x′ is apparently more stable than x.

In addition to the assumed inherent stability of the x′ factor found in Mummy it is probable that the Y factor also acts as a stabilizer of the germ plasm. The heterozygous F_1 plants of Gradus rogue × Mummy (YyXx′) have a germ plasm which is certainly less affected by the presence of the X factor than is the heterozygous Xxyy germ-plasm of Gradus rogue. With the Xx′ combination, recurring somatic mass mutation to the extent obtaining in the F_1 generation of Gradus type × Gradus rogue, does not occur. The various factors that entered the zygote take part in gamete formation at maturity and give rise to the expected F_2 combinations. Although Y behaves as a stabilizer of the F_1 germ plasm and retards mass mutation of x′ to X, the extent of the influence is difficult to determine. Nor is it known to what extent, if any, senility affects the relation of the X and x′ factors. It is possible that, with increasing age, the F_1 plants exhibit a change in the mutability of x′ to X, resulting in an increase of rogues among the F_2 segregates. Since other disturbing causes than somatic mutation in the F_1 hybrid may combine to upset the expected number or ratio of F_2 recombinations, the deviations of the observed number from the calculated number of variates in any one class can not be used, for the F_2 data at hand, to approximate the rate of change of x′ to X.

That changes in the X factor may occur in the F_1 hybrids here discussed is indicated by the exceptional F_1 plant (no. 9.727–6), which was similar in appearance and genetic behavior to the F_1 hybrids of Gradus type × Mummy. Additional evidence of mutation of X to some other form is shown in the behavior of two roguelike F_1 plants (No. 9.722–1 and No. 9.722–5). These plants apparently produced very few, or no, rogues in the next generation (families 0.1059 and 0.1093). The primary mutation or mutations of X in the F_1 parents was here delayed until late in development and, while not affecting the soma, radically changed the genetic character of the microspores and macrospores.

An analysis of the F_3 generation of the cross Gradus rogue with Mummy substantiates the hypothesis of Mendelian inheritance of the rogue factors complicated by recurring somatic mutation. The number of segregating to nonsegregating F_3 families is in close agreement with the theoretical number of 1 XX : 2 Xx′ F_2 rogue segregates expected from a monohybrid F_1 generation. The ratio of XX to Xx′ plants in the progenies of heterozygous F_2 rogues is disturbed by the phenomenon of recurring somatic mutation.

The discrepancy between observation and theory is great in respect to the ratio of rogues to nonrogues in the segregating F_3 families. However, the fact of segregation is considered more important than the ratios obtained. The instability of the heterozygous Xx′ combination, though not leading to a complete elimination of the x′ gametes, by mutation of x′ to X, is such as to produce an excess of X gametes over the number that would normally be formed if somatic mutation did not take place.

SUMMARY

(1) The Gradus variety of *Pisum sativum*, characterized by broad, wavy, emarginate stipules with a mean ratio of $\frac{\text{length of stipule}}{\text{width of stipule}}$ of 1.70, occasionally produces rogue mutations, characterized by narrow, flat, pointed stipules with a mean ratio of 2.35.

(2) Primary rogues are produced from Gradus types by a mutation of a single factor x to X. They are therefore heterozygous, of the formula xX. They give an F_2 of homozygous secondary rogues, XX, through recurrent (mass) somatic mutation of the x factor to X. Such recurrent mutation is believed to occur because of the instability of the combination of xX.

(3) The crosses Gradus × Gradus rogue, and reciprocal, likewise produce an unstable F_1 germ plasm in which mass, somatic mutation of x to X occurs.

(4) The effect of mass somatic mutation in the F_1 hybrid is to produce a germ plasm homozygous for the X factor in by far the greater number of the somatic cells. At gametogenesis only an occasional x gamete is produced. The F_2 generation consists almost entirely of rogues (XX), the Gradus type (xx) very rarely appearing.

(5) In addition to the x factor a second factor y for stipule shape is present in both Gradus and Gradus rogue. The factor y is linked with the a factor for white flowers.

(6) The English Mummy pea, a nonrogue producing variety, has a stipule ratio of 2.05 determined by a factor Y, allelomorphic to y of Gradus, and linked with the A factor for colored flowers.

(7) In crosses between Gradus and Mummy the number of cross-overs between AY and ay is approximately 20 per cent.

(8) Mummy, in addition to the Y factor, has a factor x′ which is allelomorphic to the x factor in Gradus type, therefore to the X factor of Gradus rogue. The x′ factor is practically identical with the x factor in its expression but differs in that it only rarely mutates to X, even in the combination x′X.

(9) The germ plasm of the F′ cross, Gradus rogue (yyXX) × Mummy (YYX′x′) is fairly stable. In the F′ hybrids, YyXx′, the factor x′ mutates to X but not to such an extent as to prevent at gametogenesis the formation of the usual gametes, XY, Xy, x′X, and x′y, as indicated by the appearance in the F_2 generation of rogues (having the X factor), Graduslike plants (having the x′ and y factors), and Mummylike segregates (having the x′ and Y factors).

(10) The expected 3 : 1 ratio of rogues to nonrogues in the F_2 and succeeding generations obtained from the heterozygous Xx′ plants, is disturbed by an excess of X gametes and consequently of XX and Xx′ zygotes.

(11) Inheritance of the factors for stipule shape X and y in Gradus rogue is Mendelian. Normal inheritance is obscured by somatic mutation of the factors x and x′ when in the Xx or Xz′ combination.

(12) In addition to determining a stipule ratio of 2.05 in Mummy, the factor Y acts as a stabilizer of the germ plasm. Somatic mutation of x′ to X in the F_1 cross between Mummy and Gradus rogue (Xx′Yy) does not take place to the extent in which mutation of x to X occurs in the F^1 crosses between Gradus type and rogue (XxYy) on account of the presence of the Y factor.

(13) Besides mutations of x and x′ to X, other mutations of x and x′ occur which affect stipule shape. The nature of the latter kind of mutations has not yet been investigated.

LITERATURE CITED

(1) Bateson, W., and Pellew, Caroline.
1915. On the genetics of "rogues" among culinary peas (pisum sativum). *In* Jour. Genetics, v. 5, p. 13–36, pl. 8–13.

(2) ———
1916. Note on an orderly dissimilarity in inheritance from different parts of a plant. *In* Proc. Roy. Soc. Ser. B, v. 89, p. 174–175.

(3) ———
1920. The genetics of "rogues" among culinary peas. (pisum sativum). *In* Proc. Roy. Soc. Ser. B, v. 91, p. 186–195.

(4) Brotherton, Wilber, Jr.
1920. The heredity of "rogue" types in garden peas. *In* 21st Ann. Rpt. Mich. Acad. Sci., 1919, p. 263–279, fig. 3, pl. 7–13.

(5) Emerson, R. A.
1914. The inheritance of a recurring somatic variation in variegated ears of maize. *In* Amer. Nat., v. 48, p. 87–115, 3 fig.

(6) Haldane, J. B. S.
1919. The probable errors of calculated linkage values, and the most accurate method of determining gametic from certain zygotic series. *In* Jour. Genetics, v. 8, p. 291–297.

(7) Hoshino, Yuzo.
1915. On the inheritance of the flowering time in peas and rice. *In* Jour. Col. Agr. Sapporo, Japan, v. 6, p. 229–288, pl. 12–16.

(8) Mendel, Gregor.
1866. Versuche über pflanzen-hybriden. *In* Abhandl. Naturf. Ver. Brünn, Bd. 4, (1865), p. 3–47.

(9) Morgan, T. H., and Bridges, C. B.
1919. The origin of gynandromorphs. 122 p., 70 fig., 3 col. pl. Washington, D.C. Literature, p. 116–122. (Carnegie Inst. Wash. Pub. 278, Part I.)

(10) Muller, Hermann J.
1918. Genetic variability, twin hybrids and constant hybrids, in a case of balanced lethal factors. *In* Genetics, v. 3, p. 422–499. Literature cited, p. 497–498.

(11) Tedin, Hans.
1920. The inheritance of flower colour in pisum. *In* Hereditas, Bd. 1, p. 68–97, 2 fig., 1 col. pl. Literature cited, p. 97.

(12) Tschermak, Erich von.
1911. Ueber die vererbung der blütezeit bei erbsen. *In* Verhandl. Naturf. Ver. Brünn, Bd. 49 (1910), p. 169–191, 9 fig. Bibliographical footnotes.

(13) Vries, Hugo de.
1905. Species and varieties; their origin by mutation. xviii, 847 p. Chicago.

(14) White, Orland E.
1917. Inheritance studies in pisum – iv. interrelation of the genetic factors of pisum. *In* Jour. Agr. Research, v. 11, p. 167–190. Literature cited, p. 178–180.

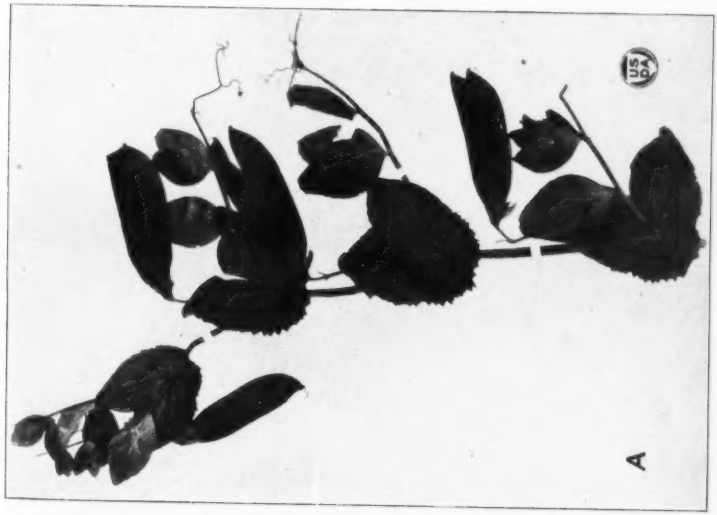
(15) ———
1917. Studies of inheritance in pisum ii. the present state of knowledge of heredity and variation in peas. *In* Proc. Amer. Phil. Soc., v. 56, p. 487–588. Bibliography, p. 583–588.

(16) Yule, G. Udny.
1916. An introduction to the theory of statistics. 382 p. London.

PLATE 1

A.—Upper part of a mature type plant of Gradus. × ¼.
B.—Upper part of a mature rogue plant of Gradus. × ¼.

B


A

C

B

A

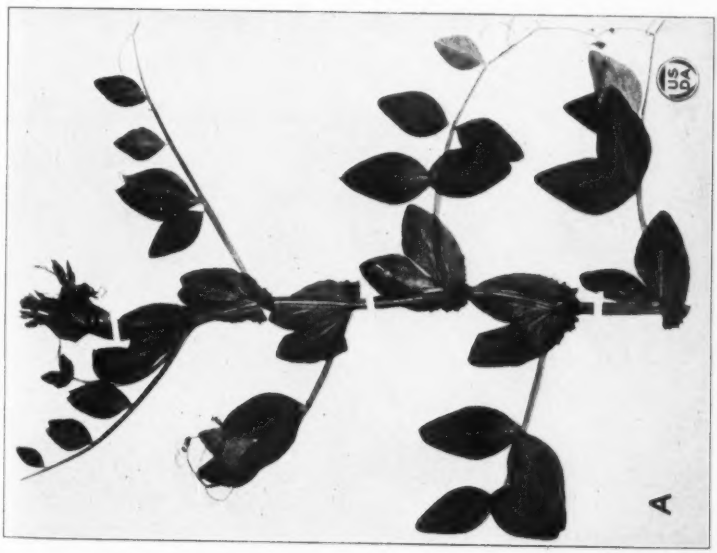
PLATE 2

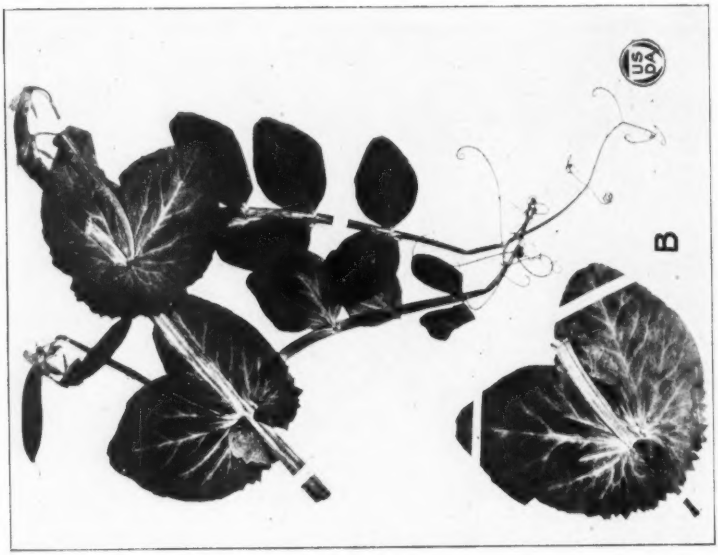
A mature plant of the Mummy variety (P16).
A.—The tip. $\times 1/5$.
B.—The part immediately below the portion shown in A. $\times 1/5$.
C.—Lower part of the stem. $\times 1/5$.

PLATE 3

A.—Upper part of a branch of a mature F_1 plant of Gradus type × Mummy. × ¼.
B.—Upper part of a branch of a mature F_1 plant of Gradus rogue × Mummy. × ¼.

B


A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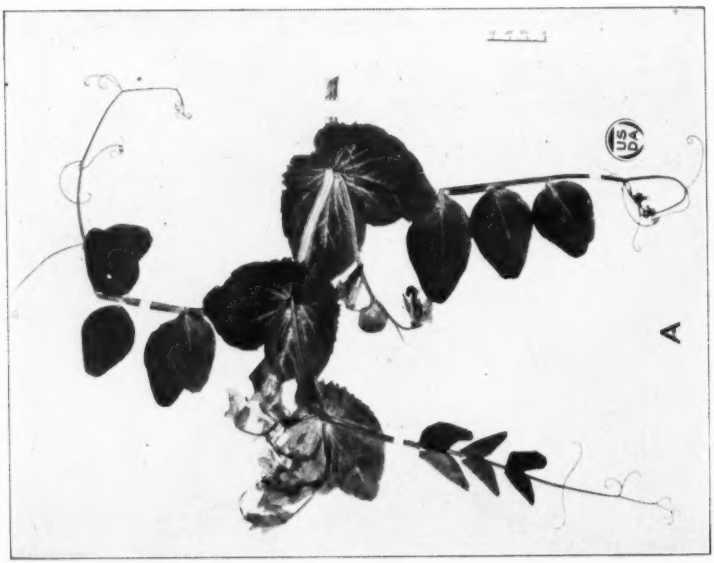
A

PLATE 4

A.—Upper part of a mature broad (y) segregate from the F_2 generation of Gradus type × Mummy. Note the typelike emarginate stipules. × ¼.
B.—The part immediately below the portion shown in A. × ¼.

PLATE 5 [a]

A.—Upper part of an intermediate (Y) or Mummylike segregate from the F_2 generation of Gradus type × Mummy. Note the narrow pointed stipules. × ¼.
B.—The part immediately below the portion shown in A. × ¼.

[a] Plates 4 and 5 are of plants from the same F_2 family.

USDA
B

USDA
A

B

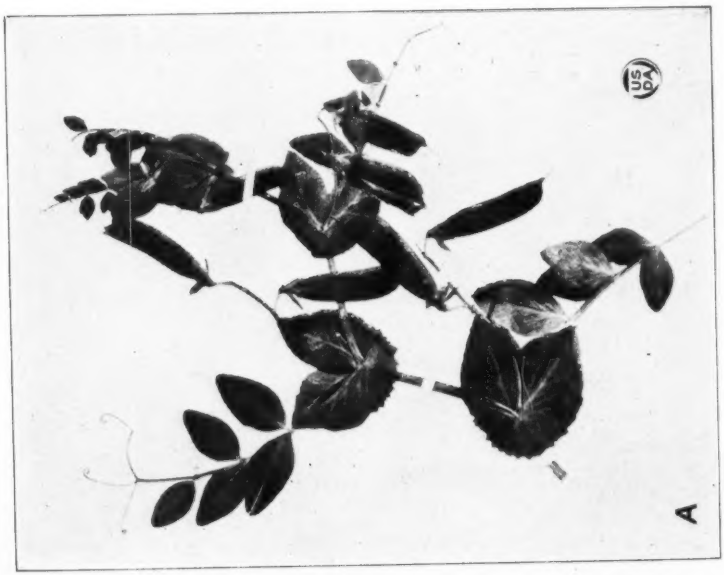
A

PLATE 6

A.—Upper part of a mature broad segregate from the F_2 generation of Gradus rogue × Mummy. × ¼.
B.—The part immediately below the portion shown in A. × ¼.

PLATE 7

A.—Upper part of a mature intermediate segregate from the F_2 generation of Gradus rogue × Mummy. × ¼.
B.—The part immediately below the portion shown in A. × ¼.

B

A

B

A

PLATE 8 [b]

A.—Upper part of a mature rogue segregate from the F_2 generation of Gradus rogue × Mummy. × ¼.
B.—The part immediately below the portion shown in A. × ⅓.

[b] Plates 6, 7, and 8 are of plants from the same F_2 family.

A METHOD OF TREATING MAIZE SEED TO DESTROY ADHERENT SPORES OF DOWNY MILDEW[1]

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POSSIBLE INTRODUCTION OF ORIENTAL DOWNY MILDEWS

Introduction into the United States of the Sclerospora downy mildews, which attack maize and related crops in the Orient, would be especially disastrous. These diseases are by nature essentially destructive and cause tremendous losses in the Orient. According to all available information they would prove equally destructive in the southern portion of our own corn belt.

Despite the dangerous possibility of thus introducing such diseases, there is a fundamental need of importing maize, not in commercial quantities, but in small quantities for experimental purposes. As the extensive maize breeding of Mr. G. N. Collins, of the Office of Crop Acclimatization and Adaptation Investigations, has shown, there may be obtained from the peculiar types of maize growing in remote countries valuable characters which can be combined most advantageously with those of our own varieties.

Under the Federal Horticultural Board regulations (including Quarantines 21, 24, and amendments), which thus far have prevented the importation of these mildews, the procedure of introducing foreign varieties of maize and related Gramineae from infested countries is necessarily difficult. As is requisite, such seed is inspected, given such treatment as any insect pest or disease found on it may demand, and grown under constant observation in an isolated quarantine greenhouse. Seed from healthy plants thus grown may then be planted without restriction by the experimenters to whom the original shipment was consigned. This procedure, although reasonable and necessary, is time-consuming and laborious.

A method of treating maize seed which will eliminate with absolute certainty any possibility of introducing maize mildews on such seed would be highly desirable. It is the purpose of this paper to present a method which the writer, after considerable experiment, has found to meet these requirements.

The oriental downy mildews of maize and related Gramineae all belong to the genus Sclerospora of the phycomycetous order Peronosporales. Several species are involved, but in the main features of structure and reproduction they agree. Through the work of Raciborski (*9*),[2] Rutgers (*11*), and Palm (*8*) on the Javan maize mildew; of Butler (*2*) on the maize mildew of India; of Lyon (*5*, *6*), Miyake (*7*), and Lee (*4*) on the maize

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[2] Reference is made by numbers (italic) to "Literature cited," p. 859-860.

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and sugar-cane mildew of Queensland, Fiji, Formosa, and the Philippine Islands; and of Reinking (*10*) and Weston (*14, 15, 16*) on the maize mildews of the Philippines, our knowledge of these forms is fairly complete. We know that if they should be carried to the United States on maize seed, it would be by resistant mycelium within the seeds, or by conidia or oospores on them. That mycelium should be the means is most improbable. Palm's work on the Javan maize mildew and unpublished experiments of the writer on the Philippine mildew of maize show that in the case of these two Sclerosporas at least, mycelium, although frequently penetrating the seed of badly infected ears, apparently is unable to transmit infection to the seedling developing therefrom. In such vegetative propagative parts as cuttings of sugar cane, living mycelium of Sclerospora may be carried and transmitted; but, in the case of maize, which is propagated only by seed, this difficulty is avoided.

Conidia adherent to maize seed can not accomplish infection. In the Philippine maize mildews the writer has found that conidia do not survive drying even for as short a time as one hour. Butler (*2*) in India and Miyake (*7*) in Formosa find this to be true of the maize mildews of those countries also.

It is preeminently probable that oospores on or in the maize seed can transmit infection. In the case of the closely related downy mildew of millet (*Sclerospora graminicola* [Sacc.] Schroet.) there is every evidence that intercontinental spread has been accomplished in this way. Whether the same means of distribution operates in the maize Sclerosporas we do not know. It should be noted in this connection that, although none of the several oriental mildews of maize has ever been found to produce oospores on maize itself, nevertheless these resting spores may occur abundantly on related hosts from which they can reach adjacent maize plantings.

Since the conidia are so easily killed, it will be by the resistant oospores, if at all, that these downy mildews will be introduced on maize seed. Consequently seed treatment must be directed against the oospores. To be effective in the case of Sclerospora diseases, a method of seed treatment not only must destroy any of these resistant spores which may be present but also must meet certain unusual requirements. The efficacy of the usual types of seed treatment is customarily demonstrated by the failure of adherent spores to give growth of the disease-producing fungus when the treated seeds have been placed in suitable media. But this would prove no index in the case of Sclerospora, as germination of the oospores has never been seen, and growth of the fungus on artificial media has never been secured. To be successful for Sclerospora, then, a method must give conclusive visible evidence of the destruction of the oospores.

EXPERIMENTS IN SEED TREATMENT

With this end in view, experiments were made with the substances customarily used in seed treatment. Of these, concentrated sulphuric acid fulfills the requirements most successfully, since it gives visible evidence of oospore destruction and yet does not impair seed germination. This was determined as follows:

Oospores (both fresh and two years dried) of the Sclerospora species so common (*14, 15*) on *Saccharum spontaneum* L. and *Miscanthus japonicus* (Thunb.) Anders. in the Philippines were mounted on a slide and carefully observed through the microscope while a drop of concentrated

sulphuric acid was drawn under the cover glass. Immediately the spores swelled and became distorted, either bursting at once or else less violently extruding oil drops and protoplasmic content while the wall softened and split open. Both content and wall rapidly darkened. Addition of India ink to the acid made more noticeable the extrusion of even small amounts of spore content. After five minutes all the spores in the field were obviously collapsed and exploded, or otherwise gave every evidence of being killed. The effect of first wetting the spores with alcohol was tried, a drop of alcohol being drawn under the cover of a dry mount, left about one minute, drawn off, and replaced by acid. In this case, the extrusion of the contents and the killing of the spores were even more rapid and complete. Water as a wetting agent was less effective than alcohol. There apparently was no difference between the wetting action of 80 to 95 per cent ethyl, methyl, or even denatured alcohol, or between the destructive effects of chemically pure or commercial concentrated sulphuric acid.

Experiments were next made to determine what effect this spore-destroying treatment would have on maize seed. Dry seeds of typical dent, flint, sweet, pop, and waxy-endosperm varieties of maize were put in separate glass jars, covered with concentrated sulphuric acid, and stirred occasionally with a glass rod during the 5 to 20 minutes of treatment. The acid was then drained off, and the seed washed for one hour in running water. Half of each lot of seed was planted, its germination recorded, and the character of the resulting plants observed. The other half was carefully dried, and its keeping quality tested by planting experimental lots from time to time. Although no quantitative results were obtained, it was found that even after the most severe treatment the viability was retained by a considerable percentage of the seed for as long a period as three months, even under the unfavorable rainy season conditions of the Philippines.

To prevent the persistence of minute air bubbles that might protect occasional spores from the acid, similar lots of seed were first given a preliminary wetting with alcohol for about a minute, the alcohol drained off, and the acid added. No difference either in immediate wetting effect, in subsequent action of acid on the seed, or in ultimate germination of the seed was apparent when 80 to 95 per cent ethyl, methyl, or denatured alcohol was used. Also there was no apparent difference between the effect of chemically pure or commercial concentrated sulphuric acid. As a result of the action of the acid, especially after treatments of 15 to 20 minutes, the surface of the seed became somewhat blackened, but the germination percentage was not decreased beyond practicable limits by even the longest treatments. Fresh acid was used for each lot of seed for fear that the black gelatinous material remaining in the acid might dilute it sufficiently to decrease its efficiency.

Some of these experiments in which representative kinds of maize were used are summarized in Table I. Calamba yellow flint is a typical example of the flinty types grown by the Christian Filipinos quite generally throughout the Philippines, Boone County White represents the American dent corn that has been introduced and successfully grown on large haciendas in several parts of the islands, while Manobo waxy is typical of the small, rapidly maturing varieties of maize cultivated by some of the non-Christian tribes in the mountains.

TABLE I.—*Effect of treatments with sulphuric acid on germination and growth of three types of maize*

Lot.	Number of kernels.	Treatment.[a]			Growth after 4 days.					Growth after 10 days.					Growth after 16 days.					Yield after 3½ months.			
					Number of plants.			Average height.	Number of leaves.	Number of plants.			Average height.	Number of leaves.	Number of plants			Average height.	Number of leaves.				
		Alcohol.	H_2SO_4	Planted, 1919.	Total.	Normal.	Abnormal.[b]			Total.	Normal.	Abnormal.[b]			Total.	Normal.	Abnormal.[b]			Plants.	Ears.	Nubbins.	Barren.
					CALAMBA YELLOW FLINT																		
		Minutes. Ethyl, 85–95 per cent.	*Minutes.* Commercial concentrated.					*Inches.*					*Inches.*					*Inches.*					
1a	40	0	5	May 24.	28	26	2	2	2–3	32	26	6	6	3–5	32	30	2	9	5–6	18	6	12	0
1b	40	½	5	...do.....	34	28	6	2	2–3	34	34	0	6	4–5	34	34	0	9	5–6	20	4	5	11
2a	40	0	10	...do.....	2	2	0	1½	2	4	2	2	5	3–4	4	4	0	6	4–5	2	1	0	1
2b	40	½	10	...do.....	28	16	12	1½	2–3	28	28	0	5	4	28	10	0	6½	5–6	22	10	12	0
3a	40	0	15	...do.....	32	26	6	1½	2–3	32	32	0	5½	4–5	32	32	0	9	5–6	15	3	7	5
3b	40	½	15	...do.....	20	14	6	1½	2–3	22	18	4	5	3–4	22	18	4	8	5–6	6	1	0	5
4a	40	0	20	...do.....	4	.....	.....	(c)	.....	14	2	12	2½	3	14	2	12	5	4–5	7	1	3	3
4b	40	½	20	...do.....	1	.....	.....	(c)	.....	4	4	0	4	3–4	4	4	0	5	4–5	1	0	1	0
A	20	.....	.....	...do.....	20	20	0	1½	2	20	20	0	6½	5	20	20	0	12	7	12	8	4	0
B	20	½	.....	...do.....	20	19	1	2	2–3	20	20	0	6½	4–5	20	20	0	12	6–7	10	7	2	1
C	20	1	.....	...do.....	20	17	3	1½	2–3	20	20	0	6	4–5	20	20	0	11	6–7	5	3	1	1
D	20	5	.....	...do.....	19	19	0	2	2–3	19	19	0	6	4–5	19	19	0	12	6–7	9	5	2	2
			Chemically pure concentrated.																				
4c	40	0	20	May 28.	12	8	4	1	2	16	10	6	4	3–4	.....	.....	.....	.....	.....	15	12	2	1
4d	40	½	20	...do.....	22	12	10	1	1–2	22	18	4	3½	3–4	.....	.....	.....	.....	.....	18	16	0	2
		Denatured, full strength.	Commercial concentrated.																				
1c	10	0	5	Nov. 23.	8	8	0	1	2	8	8	0	3½	3	.....	.....	.....	.....	.....	6	3	0	3
1d	10	½	5	...do.....	7	4	3	1	2	6	3	a 3	4	3	.....	.....	.....	.....	.....	4	2	2	0
2c	10	0	10	...do.....	6	6	0	1	2	6	5	1	3	3	.....	.....	.....	.....	.....	4	2	1	1
2d	10	½	10	...do.....	8	8	0	1	2–3	8	8	0	3	3	.....	.....	.....	.....	.....	6	1	1	4
BB	10	½	0	...do.....	.....	.....	.....	.....	.....	8	8	0	3	3	.....	.....	.....	.....	.....	7	2	3	2

BOONE COUNTY WHITE DENT

		Ethyl, 85-95 per cent.																				
1a	40	0	5	May 24..	24	20	4	1	2	26	24	2	4½	3-4	26	24	2	6	4-5	11	8	3	0
1b	40	½	5	...do....	16	8	8	1	2	18	16	2	4	3-4	18	16	2	6	4-5	8	4	4	0
2a	40	0	10	...do....	8	.....	.....	(c)	.....	12	10	2	3½	2-3	12	10	2	4½	4	4	2	2	0
2b	40	½	10	...do....	4	4	0	1	1	6	6	0	3½	2-3	6	6	0	4	4	2	2	0	0
3a	40	0	15	...do....	10	4	6	1	1	14	12	2	4	2-3	14	12	2	6	4-5	6	3	2	1
3b	40	½	15	...do....	4	4	0	1	1	8	8	0	4	3	8	8	0	6½	4	5	0	3	2
4a	40	0	20	...do....	24	12	12	1	1-2	26	24	2	4½	3-4	26	24	2	6	4-5	12	7	3	2
4b	40	½	20	...do....	30	14	16	1	1-2	28	26	2	4	3-4	28	28	0	6	4-5	15	6	6	3
A	20	.....	.....	...do....	18	17	1	1½	1-2	20	20	0	6½	3-4	20	20	0	12	5-6	12	7	1	4
B	20	½	.....	...do....	20	20	0	1	1	20	20	0	6½	4	20	20	0	12	5-6	13	4	7	7
C	20	1	.....	...do....	19	19	0	1	1-2	19	18	1	6	4	19	18	1	12	5-6	8	3	4	1
D	20	5	.....	...do....	19	19	0	1	1-2	19	19	0	6	4	19	19	0	12	5-6	7	2	1	4
			C. P. concentrated.																				
4c	40	0	20	May 28..	20	16	4	1	1-2	22	20	2	3	3-4	.....	.....	.....	.....	.....	14	8	1	5
4d	40	½	20	...do....	16	8	8	1	1-2	16	8	8	3	3-4	.....	.....	.....	.....	.....	10	2	2	0

MANOBO WAXY

		Denatured, full strength.	Commercial concentrated.																			
3b	40	½	15	...do....	12	6	6	1	1-2	6	6	e 0	2	2-3	.....	.....	.....	.....	.....	4	1	3	0
4b	40	½	20	...do....	6	2	4	1	1	4	4	f 0	2	2	.....	.....	.....	.....	.....	3	2	1	0
B	20	½	0	...do....	19	19	0	1½	2	19	19	0	5	4-5	.....	.....	.....	.....	.....	13	11	2	0

a After treatment all lots were washed in running water for one hour and were then planted immediately.
b Under "abnormal" plants are included those showing distortion, discoloration, or other evidences of atypical growth. It should be noted, however, that such growth results not only from chemical injury, as from acid, but also from mechanical injury, from flooding, or from attack by ants or other insects.
c Plants just emerging at end of 4-day period.
d One abnormal plant died.
e Three abnormal plants died.
f Two abnormal plants died.

Table I is significant only to the extent of showing that the method gives practical yields even after treatment more than rigorous enough to insure destruction of adherent spores. Too many extraneous factors are involved to allow significant comparison of such points as percentage of germination after different treatments or for different varieties. For example, the dryness and age of the seed were not in all cases the same, the location of the plots did not insure equal pollination, and the season of May to September, 1919, when most of the trials were made, terminated in six weeks of successive and violent typhoons that beat down and destroyed many plants.

From a large number of such experiments the following routine for the treatment of maize seed was formulated.

METHOD OF TREATING MAIZE SEED TO DESTROY ADHERENT SCLEROSPORA SPORES

(1) Wet the seed with alcohol for one half to one minute; drain, and, while the seed surface is still damp—

(2) Cover with concentrated sulphuric acid, allow to remain 5 to 10 minutes, stirring from time to time, then drain off the acid.

(3) Wash for one hour in running water, stirring the seed occasionally to insure complete removal of the acid.

(4) Plant at once, or dry thoroughly and save for future planting.

After carefully trying all phases of this method of treatment the writer is convinced that it meets successfully the requirements of the situation earlier outlined in this paper. By it oospores of Sclerospora that may be adhering to the maize seed may be destroyed, while at the same time the vitality of the seed is but little impaired. If planted at once, the percentage of germination is only slightly reduced, and most of the resulting plants are normal in growth and seed production. If dried and preserved, the percentage of germination declines gradually, but even after three months a sufficient number of normal plants will result to meet all practical purposes.

Preliminary experiments indicate that this method of treatment is equally effective against spores of the Physoderma disease of maize, which in the Orient involves two causal species (*3, p. 114; 12, p. 245–247*), one of them already introduced and destructive in the southern United States (*13*). Preliminary experiments have shown also that this treatment can be used successfully in the case of teosinte (*Euchlaena luxurians* Schrad.), coix (*Coix lachryma-jobi* L.), and some varieties of sorghum (*Andropogon sorghum* [L.] Brot.).

In its essential feature, i. e., the use of concentrated sulphuric acid, this method is by no means new. This reagent has been employed by several investigators for sterilizing the surface of many different varieties of seed. Brigham (*1*), for example, has used sulphuric acid to free popcorn seeds from mold spores customarily adherent to them; and the same reagent is used by the Federal Horticultural Board inspectors to destroy anthracnose spores on cottonseed. As modified to meet the exacting requirements of maize seed suspected of carrying Sclerospora, however, the method, it is hoped, will fill a peculiar need, enabling experimental quantities of seed of desirable varieties to be imported from quarantined regions afflicted with Sclerospora mildews, and insuring that, after treatment, the seed may be planted immediately without restriction and without danger.

SUMMARY

In various parts of the Orient there are several downy mildews (Sclerospora spp.) that cause severely destructive diseases of maize. Yet it is desirable that seed of varieties with useful characters be imported even from such regions for experiment and breeding. This involves the danger of introducing these dreaded diseases into our own country by oospores adhering to the maize seed. Therefore such importations are grown in quarantine greenhouses to produce seed under constant observation, a costly and time-consuming procedure. There is need of some method of seed treatment which with absolute certainty will destroy any oospores which may be adherent, and make possible immediate and unrestricted planting of the seed. Such a method not only must give conclusive visible evidence of destroying the resistant Sclerospora oospores which might be on the seed, but also must leave the viability of the seed unimpaired. The writer finds that treatment with sulphuric acid after preliminary wetting with alcohol successfully fulfills these requirements. Details of the method are given in this paper.

LITERATURE CITED

(1) BRIGHAM, R. O.
1916. STERILIZATION OF POPCORN. *In* Rpt. Mich. Acad. Sci. 17, 1915, p. 190–193. Literature, p. 192–193.

(2) BUTLER, E. J.
1913. THE DOWNY MILDEW OF MAIZE (SCLEROSPORA MAYDIS (RAC.) BUTL.). *In* Mem. Dept. Agr. India, Bot. Ser., v. 5, p. 275–280, pl. 8–9 (1 col.).

(3) IDETA, Arata.
1914. HANDBUCH DER PFLANZENKRANKHEITEN JAPANS. Ed. 4 enl., 936 p., illus., 24 pl. (8 col.). Tokyo. 1909–11. Text in Japanese; indexes and bibliography (7 p.) in German, etc. Added title-pages in Japanese, English, and French. A second Japanese t.-p., states that this is ed. 6, 1914.

(4) LEE, H. A., and MEDALLA, M. G.
1921. LEAF STRIPE DISEASE OF SUGAR CANE IN THE PHILIPPINES. *In* Science, n. s., v. 54, p. 274–275.

(5) LYON, H. L.
1911. NOTES ON THE SUGAR INDUSTRY OF FIJI. *In* Hawaiian Planters' Rec., v. 4, p. 318–339, 6 fig.

(6) ———
1915. THE AUSTRALIAN LEAF STRIPE DISEASE OF SUGAR CANE. *In* Hawaiian Planters' Rec., v. 12, p. 257–265, 2 fig.

(7) MIYAKE, Tsutome.
1911. ON A FUNGUS DISEASE OF SUGAR CANE CAUSED BY A NEW PARASITIC FUNGUS, SCLEROSPORA SACCHARI T. MIY. Rpt. Sugar Exp. Sta. Govt. Formosa, Div. Path. Bul. 1, 61 p., 9 pl. In Japanese.

(8) PALM, Bj.
1918. ONDERZOEKINGEN OVER DE OMO LIJER VAN DE MAIS. (With an English summary.) *In* Meded. Lab. Plantenziekten [Batavia], no. 32, 78 p., 7 pl.

(9) RACIBORSKI, M.
1897. LIJER, EINE GEFÄHRLICHE MAISKRANKHEIT. *In* Ber. Deut. Bot. Gesell., Jahrg. 15, p. 475–478, 4 fig.

(10) REINKING, O. A.
1918. PHILIPPINE ECONOMIC-PLANT DISEASES. *In* Philippine Jour. Sci., s. A., v. 13, p. 165–274, fig. 1–42, pl. 1–22.

(11) RUTGERS, A. A. L.
1916. DE LIJER-ZIEKTE DER MAIS. (DE PERONOSPORA-ZIEKTE DER MAIS.). *In* Meded Lab. Plantenziekten, no. 22, 30 p., 7 pl. (3 col.).

(12) SYDOW, H., SYDOW, P., and BUTLER, E. J.
1912. FUNGI INDIAE ORIENTALIS. PART IV. *In* Ann. Mycol., v. 10, p. 243–280, 11 fig.

(13) TISDALE, W. H.
1919. PHYSODERMA DISEASE OF MAIZE. *In* Jour. Agr. Research, v. 16, p. 137–154, 1 fig., pl. A–B (col.), 10–17. Literature cited, p. 154.
(14) WESTON, WILLIAM H., Jr.
1920. PHILIPPINE DOWNY MILDEW OF MAIZE. *In* Jour. Agr. Research, v. 19, p. 97–122, 2 fig., pl. A–B (col.), 16–25. Literature cited, p. 121–122.
(15) ———
1921. ANOTHER CONIDIAL SCLEROSPORA OF PHILIPPINE MAIZE. *In* Jour. Agr. Research, v. 20, p. 669–684, fig. 1, pl. 76–79.
(16) ———
1923. PRODUCTION AND DISPERSAL OF CONIDIA IN THE PHILIPPINE SCLEROSPORAS OF MAIZE. *In* Jour. Agr. Research, v. 23, p. 239–278, 2 fig., 10 pl. Literature cited, p. 277–278.

INFLUENCE OF THE SUBSTRATE AND ITS HYDROGEN-ION CONCENTRATION ON PECTINASE PRODUCTION [1]

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INTRODUCTION

Former investigations by the authors (*9*)[2] have shown that *Rhizopus tritici* Saito produces a substance of the nature of an enzym which has the power to dissolve the middle lamellae of raw sweet-potato (*Ipomoea batatas*) disks. This macerating principle was found to be thermolabile and to be produced abundantly in sweet-potato decoction in cultures one or two days old. It was found that while the mycelium itself retained some of the enzym which is called pectinase in these investigations, a portion of it was excreted into the culture solution on which it grew. Investigations have also proved that pectinase is produced by a number of other species of Rhizopus, some of which are able to cause a typical softrot of sweet potatoes. The amount of enzym produced was not equal in all cases. The most parasitic species did not necessarily produce the largest amount of enzym under cultural conditions. For example, *Rhizopus nigricans* Ehrb., which is the common softrot-producing organism in sweet-potato storage houses, produced a small amount of enzym in culture.

In order to study the action of the enzym on raw sweet-potato disks, the organism was grown for two or three days in a solution of sweet-potato decoction. The mycelium was then removed and treated according to a method described elsewhere (*9*). The ability of the enzym, contained both in the mycelium and in the solution, to macerate raw disks was tested. Measured portions of the solution were pipetted into small flasks, some of which were steamed to inactivate the enzym. Raw sweet-potato disks 1 cm. in diameter and 1 mm. in thickness were dropped into the steamed and unsteamed solutions which were then held at 45° C. Maceration was usually completed in from 2 to 4 hours in the solution containing the active enzym. The disks in the steamed controls had not been acted upon in that length of time. If, however, the disks in the control solutions were examined at the end of 24 hours, they were frequently found partially or completely macerated, the cells separating along the line of the middle lamellae in a manner typical of those in active enzym solution. In searching for an answer to this curious phenomenon two possible explanations presented themselves; first, that the enzym was not actually completely inactivated, although the solutions were steamed in an Arnold sterilizer for 10 minutes; second, that some other substance was produced which acted in a manner identical with the enzym itself.

When a modified Czapek's nutrient solution was used, a solution employed quite generally in these investigations, the results were even

[1] Accepted for publication Jan. 22, 1923.
[2] Reference is made by number (italic) to "Literature cited," p. 877–878.

more striking. It was found that in the case of this solution steaming caused no change, the steamed solutions macerating as readily as the unsteamed. These results indicated that the enzym was not secreted, but that some other substance was produced which had the power to dissolve the middle lamellae.

These results indicated two possibilities; first, that the enzym was not secreted when the fungus was grown on certain substrates or only in a small amount; and, second, that although the enzym was not secreted the organisms made the solution sufficiently acid to cause maceration of raw sweet-potato disks. In view of these facts, the authors undertook to make a study of the influence of the substrate on the production of pectinase, and of the changes in acidity produced by the fungus in different media as measured in terms of hydrogen-ion concentration. It will be shown in the discussion later that investigations have enabled the authors to explain some of the curious and puzzling results with respect to the parasitism of some of the species of Rhizopus causing soft-rot of the sweet potato.

Since *Rhizopus tritici* has been employed in so many of the investigations by the writers in this particular field, it was decided to use it in these studies. *Rhizopus tritici* grows readily in culture. It is parasitic on the sweet potato and produces an abundance of pectinase. Although not as common in storage as *Rhizopus nigricans*, it lends itself to physiological studies herein outlined more readily than does the latter species.

INFLUENCE OF THE SUBSTRATE ON PECTINASE PRODUCTION

METHODS OF EXPERIMENTATION

In order to determine what influence the substrate had on the production of pectinase, 10 different media were employed as follows: Bean, prune, Irish-potato, carrot, turnip, and sweet-potato decoctions, beef bouillon and a modified Czapek's, Pfeffer's and Richard's solutions. The vegetable and fruit decoctions were prepared by using 500 gm. of the fruit or vegetable in 1 liter of water, cooking for one hour and then filtering. Measured portions of these decoctions were put in small flasks, which were then plugged and autoclaved for about 20 minutes at 15 pounds pressure. The beef bouillon was prepared according to the usual method for preparing this medium. Some modifications were made in Czapek's, Pfeffer's, and Richard's solutions. For example, the $NaNO_3$ in Czapek's solution was replaced by an equal amount of NH_4NO_3. Glucose, 50 gm. per liter, was used as a source of carbon. The substitution of NH_4NO_3 for $NaNO_3$ was made because previous experiments showed that *Rhizopus tritici* thrived better when the nitrogen was derived from ammonium nitrate. Investigations have also shown that the fungus can not utilize cane sugar, hence glucose was substituted for it.

Pfeffer's and Richard's solutions were made according to the following formulae:

	Pfeffer's.	Richard's.
KH_2PO_4	5.0 gm.	0.5 gm.
KNO_3		4.0 gm.
$MgSO_4$	2.5 gm.	0.5 gm
NH_4NO_3	10.0 gm.	10.0 gm.
$FeSO_4$	Trace.	Trace
Glucose	100 gm.	60.0 gm.
Water	1,000 cc.	1 000 cc.

The original Pfeffer's and Richard's solutions call for cane sugar, but, in view of the fact that the fungus used in these investigations does not utilize it, glucose was employed instead. All the different media were prepared in large quantities and held in flasks stoppered with cotton and covered with oiled paper until ready for use. Just previous to using, 30 cc. of the solutions were pipetted into each of fifteen 100 cc. Erlenmeyer flasks which were then autoclaved for 20 minutes at 15 pounds pressure. Ten flasks of each solution (set) were inoculated after which they were incubated at 35° C. The remaining 5 flasks of each set were held as controls.

At the end of the growth period, usually three to four days, the mycelium from all the flasks of a single series was collected into one compound sample and prepared for macerating experiments, according to methods previously described. The solutions on which the fungus grew in each set were made into one compound sample and equal portions (about 30 cc.) pipetted into small flasks to which raw sweet-potato and carrot disks were added. A portion of each of the solutions was steamed for 10 minutes to inactivate the enzym which, together with flasks of the original solutions which had not supported a fungous growth, were used as controls. An accurately weighed amount of mycelium (0.25 gm.) ground in pure quartz sand was used in determining the macerating action of the hyphae. The mycelium was not extracted in water prior to the addition of the raw disks, since previous experiments showed (9) that the rate of maceration was not influenced thereby. The ground mycelium was included in the system.

The hydrogen-ion concentrations of the solutions and of the controls (not inoculated) were determined by the electrometric method as rapidly as possible after the hyphae were removed, in all cases during the same day.

EXPERIMENTAL DATA

In this series of experiments 10 different solutions were employed, 6 being of vegetable origin, 3 synthetic solutions, and 1 of beef bouillon. In one or two series of experiments in which a modified Czapek's nutrient solution was employed as a substrate it was noted that no pectinase seemed to be produced, although it was abundantly secreted in sweet-potato decoction with which it was being compared. It was also noted in these cases that even in the absence of pectinase a certain amount of maceration of sweet-potato disks occurred. In view of these facts, experiments were undertaken to study the regulating action of certain substrates on the production of the enzym. The substrates here employed were selected as being representative of the vegetable decoctions and synthetic solutions in common use. It was also believed that if the substrate exercised any influence on the production of pectinase that fact would be brought out by these media.

MYCELIAL GROWTH

In these series of experiments the dry weight of the mycelium was not determined. The mycelium was all used in maceration experiments. In general, the growth and fruiting of the fungus on the media of vegetable origin were good, being much better than they were when the fungus was grown on the synthetic solutions or on beef bouillon. Bean, carrot, and turnip decoction yielded the best growth and fruiting. A fair growth took place in prune decoction, but little or no fruiting. The

growth and fruiting on Irish-potato decoction were good but somewhat inferior to what they were on bean, carrot or turnip. The growth was poorer on sweet-potato decoction than on any of the vegetable preparations. The growth on the three synthetic media and on beef bouillon was not as good as on the vegetable decoctions. A better growth was made on beef bouillon than on Czapek's, Pfeffer's, or Richard's solutions. The poor growth on Czapek's solution was rather surprising in view of the fact that Rhizopus usually grows well and fruits abundantly on it. The time for terminating the experiment was gaged by the stage of fruiting of the fungus. It was shown by Brown (*2*) and by Harter and Weimer (*9*) that the maximum amount of the macerating principle was contained in the mycelium just at or just preceding fruiting. Fruiting began a little earlier on the vegetable media than on the synthetic ones, consequently the experiments in which the vegetable media were used were terminated usually one day sooner than the others.

HYDROGEN-ION CONCENTRATION

The hydrogen-ion concentrations of the uninoculated solutions and the solutions on which the fungus grew are shown by Table I.

TABLE I.—*Hydrogen-ion concentration of inoculated solutions and controls (uninoculated at the end of the experiments in terms of* P_H)

Media.	Experiment 1.		Experiment 2.	
	Control.	Inoculated.	Control.	Inoculated.
String-bean decoction	4.88	7.82	4.80	7.99
Prune decoction	3.95	3.58	3.91	3.53
Irish-potato decoction	5.68	7.48	5.60	7.80
Carrot decoction	5.18	4.36	4.98	4.47
Turnip decoction	5.05	4.03	4.83	4.26
Sweet-potato decoction	5.22	3.24	5.05	3.31
Czapek's solution	4.84	2.34	4.14	2.47
Pfeffer's solution	3.53	2.56	3.48	2.61
Richard's solution	3.44	2.51	3.36	2.46
Beef bouillon	8.29	8.18	8.04	8.52

A survey of Table I shows that there is about as close an agreement in P_H values between the two experiments as could be expected from two experiments carried out at different times and under somewhat different conditions. The greatest variation noted is in the controls in connection with Czapek's nutrient solution. The solutions for the two experiments were prepared at one time so that those used for the second experiment stood for some time longer. A certain amount of evaporation would probably take place and chemical reactions are not unlikely.

A further examination of the table reveals other curious facts with respect to the hydrogen-ion concentration. The growth of the fungus caused some change in the hydrogen-ion concentration of all the solutions although in the case of beef bouillon and prune decoction it was not large. On the other hand, the change produced by the fungus when growing on string bean and Irish potato decoction and on Czapek's solution was quite marked. In experiment 1 in string-bean decoction the hydrogen-ion concentration was decreased from P_H 4.88 to 7.82 and

on Irish-potato decoction from P_H 5.68 to 7.48. The hydrogen-ion concentration was increased from P_H 4.84 to 2.34 on Czapek's solution.

If a comparison is made of the hydrogen-ion change produced in the six vegetable decoctions, it will be seen that in some cases it is increased and in others decreased. This is contrary to what might be expected on theoretical grounds. Before these experiments were conducted it had been many times demonstrated that *Rhizopus tritici* made sweet-potato decoction more acid. For this reason it was predicted that it would produce similar changes when growing on other vegetable decoctions prepared in a similar manner. However, it was found that some of the decoctions (carrot, turnip, sweet potato) became more acid while others (string bean, Irish potato) became less so.

The synthetic media were all more acid at the close than at the beginning of the experiment, Czapek's nutrient solution showing a greater increase in hydrogen-ion concentration than either Pfeffer's or Richard's solutions. Beef bouillon, a solution not well suited to the growth of *Rhizopus tritici*, was very little changed in acidity, becoming less alkaline and more alkaline in experiments 1 and 2 respectively.

The outstanding conclusion to be drawn from these experiments alone is that it is not safe to class any one fungus as an acid or an alkaline-producing organism. These experiments too clearly demonstrate that an organism may be an acid producer on one medium and the reverse on another. The class to which an organism belongs would clearly seem to depend upon the media used and should be determined in each individual case.

ENZYM PRODUCTION

The amount of enzym produced was measured by the time required to macerate raw sweet-potato and carrot disks 1 mm. thick suspended in the solution on which the fungus grew and in a water suspension of the mycelium. The time required to effect loss of cohesion of the cells of disks by the enzym secreted into the solution of the different media and retained by the mycelium is shown by Table II. It must be remembered that these results are only approximate, since it is not always easy to tell just when loss of coherence is complete. It is believed, however, that they are accurate within the limits of ± 5 per cent.

TABLE II.—*The time (in hours) required to macerate raw sweet-potato and carrot disks by the enzym in the solution and in a water suspension of the mycelium*

Media.	Experiment 1.			Experiment 2.		
	Solution (sweet potato).	Hyphae.		Solution.		Hyphae (sweet potato).
		Sweet potato.	Carrot.	Sweet potato.	Carrot.	
String-bean decoction	6.00	7.0	7 to 24	4.5	7 to 24	7 to 24.
Prune decoction	None in 24.	7 to 24		None in 24	None in 24.	7 to 24.
Irish-potato decoction	3.75	5.0	7 to 24	3.0	6.5	7 to 24.
Carrot decoction	2.75	4.5	7 to 24	3.0	3.0	5.0
Turnip decoction	2.25	4.0	5	2.0	2.0	3.0
Sweet-potato decoction	2.75	5.5	7 to 24	3.0	5.5	6.75.
Czapek's solution	None in 24.	Considerable in 30.	None in 30.	None in 24.	None in 24.	None in 24.
Pfeffer's solution	do	do	do	do	do	Do.
Richard's solution	do	do	do	do	do	Do.
Beef bouillon	do	do	do	do	do	Do.

It was demonstrated that a macerating enzym was produced by *Rhizopus tritici* when grown on all the vegetable decoctions, with the possible exception of the prune, and that this enzym was quite active in some cases (carrot, sweet potato, and turnip). An examination of experiment 2, Table II, shows that loss of coherence of both sweet-potato and carrot disks was complete in the turnip decoction in two hours and in the carrot decoction in three hours, and in the suspension of hyphae grown on these decoctions in three and five hours respectively. The rate of maceration by the enzym in the sweet-potato decoction and by that in the hyphae grown on this solution was about the same as that obtained in many previous tests. Although there was a fair growth of mycelium on prune decoction, the writers were unable to demonstrate the presence to any extent of pectinase either in the solution or in the hyphae. With this exception, the vegetable media all produced a demonstrable amount of pectinase. There was considerable variation in the amount produced in the different media, the complete loss of coherence being shown by some solutions in less than one-half the time required by others.

The production of pectinase could not be demonstrated in any of the synthetic solutions or in beef bouillon or the mycelium grown on them. From these results it seems safe to conclude that there is something in the composition of most of the vegetable decoctions which stimulates the production of pectinase, which is not present in the synthetic media or in beef bouillon.

A probable explanation of the difference between the synthetic media and the vegetable decoctions with respect to the production of pectinase by the fungus growing on them may be sought in the regulatory action of the substrate. A number of investigators have shown a quantitative regulation of the production of enzyms, while Knudson (*15*) demonstrated a qualitative regulation of tannase with *Aspergillus niger* and *Penicillium* sp. He found that these fungi produced gallic acid by the fermentation of tannic acid when the latter was added to a modified Czapek's nutrient solution, but if supplemented with glucose no tannase was formed. A number of other substances when used as a source of carbon failed to stimulate the secretion of tannase. Katz (*14*) studied the regulatory action of certain chemical substances in the substrate on the secretion of amylase by *Penicillium glaucum*, *Aspergillus niger*, and *Bacillus megatherium* and found that while the amylase secretion was not prohibited by the presence of substances chemically allied to starch, their effect was to greatly inhibit it. He found that the different fungi did not act identically and cites as proof the results obtained with *A. niger* and *P. glaucum*, in which case sugar had a much less inhibiting effect on the production of the enzym with the former than with the latter. Similar conclusions were reached by Duclaux (*6*) with *P. glaucum* and *A. glaucus*, though he considered only the enzym excreted into the culture medium. A number of other investigators, among them Kylin (*16*), have made similar studies. He worked with *P. glaucum*, *P. biforme*, and *A. niger*, and found no qualitative regulation of the enzyms (diastase, invertase, and maltase), though a quantitative regulation was conclusively proved. In the case of *P. glaucum* the regulatory secretion of diastase was greater than in that of *A. niger*. In this same connection the results obtained by Young (*26*) with *A. niger* may be cited. He showed that inulase was produced in greatest amount in the mycelium when inulin was used as the source

of carbon, but was likewise produced when other carbohydrates were employed. The substances most closely allied to inulin were most efficient in the production of the enzym. Investigations along similar lines have been made by Went (*22*), Wortmann (*24*), Dox (*5*), Pfeffer (*19*), Brunton and MacFayden (3), Harter (*8*), and others. Went, for instance, showed that *Monilia sitophila* secreted a number of enzyms, some of which were produced only when the particular substances on which they act were present in the culture solution. Brunton and MacFayden found that a bacterium formed diastase when cultivated on starch paste but not when grown on meat broth. Dox, on the other hand, demonstrated that the enzyms were secreted by *Penicillium camemberti*, regardless of the chemical nature of the substrate. He found that by cultivating the fungus on any particular substratum the quantity of the corresponding enzym could be increased, but that no enzym not normally produced by the organism could be developed by any special method of nutrition. Harter likewise showed that when different carbohydrates were used alone or in combination in the culture medium, although amylase was produced when sugars alone were employed, it was secreted in greatest abundance when starch was the only source of energy.

The preceding review of some of the literature shows that the quantitative regulation of enzyms is a rather common phenomenon. The qualitative regulation, however, has been demonstrated in only a few cases. Failure to demonstrate the presence of an enzym does not necessarily constitute positive proof that it is not secreted. It is a well known fact that some enzyms act only under certain conditions—that is, in the presence of certain acids or alkalies or electrolytes or other substances, the so-called co-enzyms. For example, it has been shown that the presence of either the chlorin or bromin ion is absolutely essential to the activity of pancreatic amylase (*1*). It is a fact, however, that the writers have been unable to demonstrate the production of pectinase by *R. tritici* on Czapek's, Pfeffer's, and Richard's solutions and on beef bouillon. On the other hand, it was freely produced in all vegetable decoctions with the exception of prune decoction, where its secretion was doubtful.

With these facts in mind the writers suspected that there might be some substance or substances in the vegetable decoctions which were stimulating the production of pectinase that were absent in the synthetic media and in beef bouillon and that this substance was probably one or more of the pectic compounds. Experiments were therefore initiated in which pectin obtained in as pure a state as it was possible to make it was introduced alone and in combination with glucose into Czapek's modified nutrient solution as the available sources of carbon.

The pectin was obtained from the carrot, the method described by Hunt (*10*) being followed for the most part in its preparation. A number of flasks were prepared, using Czapek's modified nutrient solution as the substrate. In some cases dextrose (20 per cent), in others pectin (1 per cent), and in still others dextrose (20 per cent), and pectin (1 per cent) in combination were supplied as the source of carbon. Thirty cubic centimeters of these solutions were placed in each flask and after inoculation with a spore suspension of *Rhizopus tritici* the cultures were incubated at 35° C. At the end of 4 days' growth the mycelial felts from all of the flasks of a single series were combined into one compound sample and prepared according to the usual method with acetone and ether for macerating experiments. Likewise, the solutions on which the

fungus grew from a single experiment were made into one sample and immediately used for the maceration of raw sweet-potato disks. A portion of this solution which was first steamed to inactivate the enzym together with the uninoculated solution, served as controls. Hydrogen-ion determinations were made of the inoculated solutions and of the uninoculated controls at the close of the experiment.

A good growth was obtained in each of the different series. The best growth and fruiting, however, were obtained when dextrose and pectin were combined in the same solution.

Some interesting results with respect to the hydrogen-ion concentrations were likewise obtained. In one experiment Czapek's solution plus 20 per cent dextrose with an original hydrogen-ion concentration of P_H 5.24 had a P_H of 1.93 after the fungus had grown on it for 4 days. When 1 per cent pectin alone was substituted for the dextrose the solution had an original P_H of 4.38 and (after the fungus had grown upon it) a final hydrogen-ion concentration of P_H 3.76.

In another experiment the following results were obtained: Czapek's solution plus 20 per cent dextrose with an initial P_H of 4.94 had a final P_H of 1.85. When 20 per cent dextrose and 1 per cent pectin were used the initial P_H was 4.09 and the final 1.70. If, on the other hand, pectin alone was used the original hydrogen-ion concentration was P_H 4.43 and the final P_H 3.90. The results show clearly that the pectin itself while supporting a good growth of mycelium is not so efficacious in the production of acid as dextrose.

Equally striking results were obtained with respect to the production of a middle lamellae dissolving enzym. It has already been pointed out that pectinase was not produced on Czapek's nutrient solution with dextrose as a source of carbon. Comparative experiments as detailed above showed that only a feeble macerating principle could be demonstrated either in the mycelium or in the solution on which the fungus grew when Czapek's solution plus 20 per cent dextrose was used or when the same solution plus 20 per cent dextrose plus 1 per cent pectin were employed as the source of energy. On the other hand, when pectin alone was used as a source of carbon a vigorous cell wall dissolving enzym was secreted. In one series of experiments coherence of the cells of sweet-potato disks immersed in the solution was entirely lost in 3¼ hours when pectin alone was used. When dextrose was employed 24 hours were required. There was no maceration in 24 hours in the steamed control when pectin was used. When glucose was used maceration was completed in 24 hours, which was probably caused by the acid (P_H 1.93) in the solution.

A second series of experiments gave results similar to those discussed above. When pectin alone was used as the source of carbon maceration in the solution and by means of the mycelium was completed in two and three hours, respectively. On the other hand, when glucose alone or in combination with pectin was employed 22 hours were required for the complete loss of coherence of the cells in the solution and in a water suspension of the mycelium. It is interesting to note in this connection that maceration was completed in the steamed controls in 22 hours, which may have been due to the acid (P_H 1.85 in 20 per cent dextrose and P_H 1.70 in 20 per cent dextrose + 1 per cent pectin) formed.

The results of the experiments as detailed above seem to indicate clearly a rather marked quantitative if not qualitative regulation of

pectinase. Although the qualitative regulation of pectinase has been demonstrated here for the first time, it is a well-known phenomenon for other enzyms. A brief review of some of the literature bearing on other enzyms showed that some of them, diastase for instance, are produced more abundantly when starch forms the only available source of carbohydrates in the solution.

INFLUENCE OF THE HYDROGEN-ION CONCENTRATION OF THE SUBSTRATE ON PECTINASE PRODUCTION

METHODS OF EXPERIMENTATION

Two different solutions, sweet-potato decoction and Czapek's modified solution, were used in the investigations of the influence of the hydrogen-ion concentration of the substrate on pectinase production. These two solutions were employed for two reasons: First, it has proved that *Rhizopus tritici* makes a good growth on both of them; second, they are easily prepared and thus available for experimental work at all times. The original solutions were prepared in sufficient quantity for a number of experiments. In the case of Czapek's nutrient solution the chemicals were put into a large enamel-ware vessel and the water added. The sugar was then added and the mixture heated for about one-half hour. A precipitate which formed during the process of heating was removed by filtering. The sweet-potato decoction was prepared according to the method described on page 862, except that 800 gm. of potato were used for each liter of water. From this point on the methods employed were the same for both solutions. Both solutions were made up to a strength such that when 30 cc. were diluted to 50 cc. they were of the desired concentration. The general method for adjusting a solution to a definite hydrogen-ion concentration described by Karrer and Webb (13) was employed. These authors, using Czapek's solution and beet decoction, worked out a method whereby the addition of a definite amount of acid, alkali, or water to 30 cc. of the stock preparation would give a definite hydrogen-ion concentration. They showed that it was possible to obtain a range of P_H 1.2 to 10 + and 1.2 to 9.8 in beet decoction and Czapek's solution respectively by varying the amounts of N/5 HCl and N/5 KOH. The writers, selecting out of the series given by Karrer and Webb such P_H values as they wished to use, employed their method and made up Czapek's nutrient solution and sweet-potato decoction to these hydrogen-ion concentrations. Although the hydrogen-ion concentrations obtained were not identical with those obtained by Karrer and Webb, they gave a range sufficient to meet all the needs of these experiments. The final amount of the solution in each flask was 50 cc. Exactly 30 cc. of the stock solution (Czapek's solution or sweet-potato decoction) were added to each flask by means of a self-filling burette. The desired amount of water was added and the solutions were then autoclaved for 20 minutes at 15 pounds pressure. The required amounts of acid and alkali were then added to each flask under aseptic conditions. The burette was first sterilized with 95 per cent alcohol and then washed with sterile distilled water. Care was taken at every step to prevent contamination. The quantity of acid, alkali, or water to be added to make the solutions up to 50 cc. depended upon the hydrogen-ion concentration desired.

The acid and alkali with which the dilutions were made were held in two liter flasks. The flasks were plugged with rubber stoppers through

which two glass tubes passed, one of which was long enough to extend nearly to the bottom of the flask. The liquid was drawn out through the long tube. The other tube, through which the air entered the flask, was stoppered with cotton to prevent the entrance of contaminating organisms with the inflowing air. The flasks so prepared were autoclaved for 20 minutes at 15 pounds pressure.

The culture flasks, after being finally prepared, were allowed to stand for two days before inoculation in order to determine which if any of them were contaminated. In spite of all the possible precautions, some of the flasks in every experiment became contaminated. These were discarded and a portion of the remaining flasks were inoculated with a spore suspension of *Rhizopus tritici*. Some of the flasks were held as controls. The cultures were incubated in the dark at a constant temperature of 35° C.

The duration of the experiment varied from 5 to 8 days. At the close of the experiment a portion of the mycelium and of the solution on which the fungus grew was prepared according to methods already described and used for macerating tests. Another portion of the mycelium was used in obtaining the dry weight of the fungous material produced. A portion of the solution was used to determine the hydrogen-ion concentration and the amount of dextrose present in Czapek's solution only. The sugar was determined by means of a Fric saccharimeter. The hydrogen-ion concentration and sugar content of the controls and inoculated flasks were determined on the day the experiment was terminated.

Fifteen flasks of each hydrogen-ion concentration were prepared, 10 of which were inoculated. The remaining 5 were held as controls.

EXPERIMENTAL DATA

It has already been shown that pectinase was not produced in Czapek's nutrient solution when dextrose was used as a source of carbon. It was found, however, that when pectin was employed in the substrate as the only source of carbon in Czapek's solution a powerful middle lamellae dissolving enzym was secreted. When pectin was combined with dextrose as a source of energy pectinase was produced, but its action was much slower, being presumably secreted in a much smaller amount. It was likewise pointed out that *Rhizopus tritici* produced a macerating substance on several vegetable decoctions (turnip, sweet potato, Irish potato, carrot, and bean) but not on synthetic media (Pfeffer's, Richard's, and Czapek's solution and beef bouillon). Furthermore, it has been demonstrated that some substance which causes maceration of raw sweet-potato disks is produced when *Rhizopus tritici* is grown on Czapek's nutrient solution. That this substance was an acid seems likely as some of the experiments to be detailed below will show. In view of the fact that Czapek's nutrient solution became rapidly acid when *R. tritici* was grown on it, it was suspected that the production of the acid interfered with the secretion of pectinase. It is also interesting to note in the way of comparison that when sweet-potato decoction in which pectinase was abundantly produced was used as a substrate the final acidity as measured in P_H, although increased, was never as high as in Czapek's solution.

A series of experiments were therefore outlined to determine what effect the original hydrogen-ion concentration of the substrate (Czapek's

solution, sweet-potato decoction) would have on the secretion of pectinase. The methods used in these experiments have been outlined already. The results of these experiments are shown in Tables III to VI.

Table III gives the results of experiment 1, in which the P_H values of the original control solutions (column 1) varied from 1.04 to 8.53. In this experiment the fungus grew for 5 days.

TABLE III.—*(Experiment 1) showing hydrogen-ion concentration and dextrose content of the control and inoculated Czapek's solution, the time required by them to macerate sweet-potato disks, and the dry weight of the mycelium produced*

P_H of control.	P_H of inoculated.	Dextrose in control.	Dextrose in inoculated.	Time required to cause maceration in control.	Time required to cause maceration in inoculated.	Dry weight of mycelium.
		Per cent.	*Per cent.*			*Gm.*
1.04	1.01	14.7	15.25	Complete in 5 hours	Complete in 5 hours	None.
1.71	1.76	14.95	15.25	Complete in 24 hours	Complete in 24 hours	Do.
2.62	2.27	14.95	14.55	Some in 48 hours	Some in 48 hours	0.0283
3.58	2.18	14.95	13.75	Nearly complete in 48 hours	Complete in 48 hours	.0728
5.82	2.17	14.95	13.40	do	do	.0972
6.01	2.18	14.95	13.30	Complete in 48 hours	do	.1005
6.57	2.15	14.95	13.00	do	do	.1147
7.28	2.20	14.95	12.55	do	do	.1357
7.61	2.32	14.90	12.20	Some in 48 hours	do	.1553
7.91	2.39	14.85	12.00	Very little in 48 hours	Nearly complete in 48 hours	.1620
8.12	2.59	14.70	12.40	Doubtful if any in 48 hours	Very little in 48 hours	.1748
8.53	2.66	14.55	12.40	None	Practically none in 48 hours	.1780

In experiment 2 (Table IV) the fungus was allowed to grow for seven days. Carrot disks were macerated in about the same time as the sweet-potato disks. If there was any measurable difference the carrot disks were macerated in a little less time than the sweet-potato disks.

TABLE IV.—*(Experiment 2) Showing the hydrogen-ion concentration and dextrose content of the control and inoculated solutions, the time required by them to macerate sweet-potato and carrot disks, and the dry weight of the mycelium produced*

P_H of control.	P_H of inoculated.	Dextrose in control.	Dextrose in inoculated.	Time required to cause maceration in control.	Time required to cause maceration in inoculated.	Dry weight of mycelium.
		Per cent.	*Per cent.*			*M.*
1.67	1.63	15.9	15.9	Complete in 22 hours	Complete in 22 hours	None.
2.67	2.22	15.9	14.9	Slight maceration in 30 hours	Slight maceration in 30 hours	0.0340
3.22	2.09	15.9	14.0	A little maceration in 30 hours	A little maceration in 30 hours	.0766
4.39	2.18	15.9	14.0	do	do	.0812
4.81	2.18	15.9	14.0	do	do	.0814
5.69	2.12	15.9	13.5	do	do	.1040
6.46	2.18	15.9	12.8	do	do	.1283
6.78	2.46	15.8	12.2	No maceration in 30 hours	No maceration in 30 hours	.1539
6.97	2.47	15.4	11.7	do	do	.1679
7.23	2.59	15.4	11.1	do	do	.1718
7.80	2.58	15.4	9.2	do	do	.1734

TABLE V.—(*Experiment 3*) *Showing the hydrogen-ion concentration of the control solution (sweet-potato decoction) at the beginning and end of the experiment, also of the inoculated solution, and the time required by the solution and mycelium to macerate sweet-potato disks*

P_H of control at end of experiment.	P_H of inoculated solution at end of experiment.	P_H of control at beginning of experiment.	Time required to complete maceration.			
			In inoculated unsteamed solution.	In inoculated steamed solution.	In uninoculated solution.	By hyphae ¼ gm. in 25 cc. H_2O.
			Hours.	*Hours.*	*Hours.*	*Hours.*
1.27	1.27	1.28	2	2.25	42	.....
2.05	2.10	2.10	7–24	48	48	.....
2.67	2.55	2.71	2.25	24	Slight in 48	5.0
3.80	3.12	3.83	2.00	4.5	None in 48	4.0
4.66	3.20	4.69	2.00	24.0	do	4.0
5.06	3.36	5.20	2.25	24	do	5.0
6.14	3.48	6.17	2.25	48	do	5.0
7.12	3.57	7.19	2.25	None in 48	do	.....
7.58	3.60	7.67	2.50	do	do	.....
8.28	3.69	8.33	2.50	.....	.....	5.5

TABLE VI.—(*Experiment 4*) *Showing the hydrogen-ion concentration of the uninoculated sweet-potato decoction at the beginning and end of the experiment, also of the inoculated solution, dry weight of mycelium produced, and time required to macerate sweet-potato disks*

P_H of control at beginning of experiment.	P_H of control at end of experiment.	P_H of inoculated solution at end of experiment.	Dry weight of mycelium.	Time required to complete maceration.			
				In inoculated unsteamed solution.	In inoculated steamed solution.	In uninoculated solution.	By hyphae ¼ gm. in 25 cc. H_2O.
			Gm.	*Hours.*	*Hours.*	*Hours.*	*Hours.*
1.24	1.24	1.27	.....	3.00	6	18	.....
2.01	2.01	2.02	.....	24.00	24	18	.....
2.69	2.63	2.54	0.0965	3.25	48	None in 48	4.75
3.63	3.57	3.17	.0115	2.50	24	do	3.50
4.33	4.31	3.29	.1268	3.00	24	do	3.75
5.13	5.05	3.51	.1214	3.00	24	do	4.00
6.05	6.02	3.51	.1186	3.00	24	do	4.50
7.29	6.32	3.58	.1388	3.25	48	do	4.50
7.99	7.53	3.72	.1280	3.25	None in 48	do	4.75
8.30	8.21	3.86	.1297	3.50	do	do	4.75
9.12	8.86	8.14	.0693	None in 48	do	do	5.50

In experiment 4, the fungus felts from five flasks were collected and the dry weight determined. The average weight is given in Table VI. The mycelium from the other flasks was used in determining the rate of maceration.

An examination of Tables III to VI shows some interesting facts with respect, first, to the changes in hydrogen-ion concentration of the solution produced by the fungus; second, to the influence these changes have on the production of pectinase; third, to the consumption of sugar (in Czapek's solution); and fouth, to the amount of mycelium produced. It will be seen that growth was entirely inhibited at a P_H of 1.7. The maximum limit of alkalinity for growth was not obtained, since the alkali in the solutions prepared to be about P_H 11.0 appeared to react with the sugars, thereby reducing the alkalinity. It was found in general, however, that the growth was best in the most alkaline solutions so long

as it was not alkaline enough to inhibit growth. Growth was better in sweet-potato decoction than in Czapek's nutrient solution at approximately the same hydrogen-ion concentration.

The fungus tends in all cases to make the solution more acid. The degree of acidity reached was greater in Czapek's solution than in sweet-potato decoction. In experiment 1 (Table III) no growth took place at a P_H of 1.64 and 1.71 and the hydrogen-ion concentration was therefore not much changed. As the hydrogen-ion concentrations of the control solutions decrease, however, the change in the P_H value of the inoculated solutions becomes greater, as, for example, P_H 8.53 (Table III) was changed to 2.66. It will be seen from Table III and the other tables also that the P_H of the inoculated solutions gradually increase with the decrease in the hydrogen-ion concentration of the control solutions.

That many fungi and bacteria make the substrate acid or alkaline is well known. Currie (*4*) showed that citric acid was produced by *Aspergillus niger* in a nutrient solution and Wehmer (*20*) gave the generic name Citromyces to a group of fungi which he believed was characterized by its ability to produce the same acid. Lafar (*17*), and Lind (*18*) found that oxalic acid was produced by certain species of Penicillium, Botrytis, and Citromyces. Weimer and Harter (*21*) showed that *Rhizopus tritici, Diplodia tubericola, Mucor racemosus, Penicillium sp.* and *Botrytis cinerea* increased the acidity of Czapek's nutrient solution when glucose was used as the source of carbon. Young and Bennett (*25*) found that *Fusarium oxysporum* when grown on Richard's solution with a P_H 5 first made the solution acid and then alkaline so that in 40 days a P_H of 7.4 was reached. Bacteria appear to produce acid in the solution, though not to so marked a degree. For example, Jones (*11*) found that a strain of Pneumococcus when inoculated into a medium with an initial reaction of P_H 7.0 grew poorly and developed a P_H of 6.2. If, on the other hand, the initial reaction was 7.6 a good growth and a final P_H of 5.4 resulted. *Streptococcus viridans* was found by Grace and Highberger (*7*) to change a broth with an initial hydrogen-ion concentration of between P_H 7.1 and 7.3 to a P_H 6.6 in 6 days. Similar changes in the hydrogen-ion concentrations were noted by Wolf and Harris (*23*) and by Karrer (*12*) with certain bacteria and fungi, respectively. A comparison of the results of other investigators with those obtained by the authors seems to indicate that *Rhizopus tritici* renders the substrate more acid than most fungi or bacteria. As a matter of fact, it will be seen that the degree of acidity produced (P_H 2.09, Table IV) while not prohibitive of growth, which is practically stopped in a P_H 1.7, closely approximates it.

From Tables III and IV it is seen that pectinase is not produced in Czapek's nutrient solution when adjusted to any of the hydrogen-ion concentrations tested. This conclusion is drawn from the fact that, although a certain amount of maceration took place in certain solutions, especially in those with the highest hydrogen-ion concentrations, there was just as much in the steamed as in the unsteamed solutions. In the solution with an initial P_H of 1.01 (Table III) no growth of the fungus took place and yet maceration was complete in 5 hours. When the initial P_H was 1.76, 24 hours were required to complete maceration. At this concentration no growth occurred. At all other concentrations there was a normal amount of mycelium produced and the final hydrogen-ion concentration of the inoculated solutions varied from P_H 2.15 to 2.66

(Table III). At a concentration of P_H 2.66 no maceration occurred. At the other concentrations about 48 hours were required for the cells to completely lose their coherence. There is therefore a degree of acidity such that the acid has no action on the middle lamellae in 48 hours (P_H 2.66, Table III). At a P_H of 2.59 little maceration took place in 48 hours. In the remaining solutions (Table III) the acidity is about sufficient to dissolve the middle lamellae in 48 hours. At P_H 1.76 maceration is complete in 24 hours and at 1.01 in 5 hours. Similar data are shown in Table IV.

The data presented in Tables V and VI were obtained by growing *R. tritici* on sweet-potato decoction adjusted to the different P_H values. These show that the decoction does not become as acid as Czapek's solution. No growth occurred in P_H 1.27 and 2.03 (Table VI), but at all other concentrations about the same amount of mycelium was produced. The hydrogen-ion concentration of the solutions when there was growth was considerably increased; however, in no case was the increase as great as that found in Czapek's solution. At the two higher concentrations the maceration produced was doubtless due to the action of the acid, since in one case (P_H 1.27, Table V), although there was no mycelium produced, coherence of the cells was lost in 2 hours, and in another case (P_H 2.10, Table V) in 7 to 24 hours. In all the other concentrations the middle lamellae were completely destroyed in from 2 to 2½ hours in the unsteamed solutions. In the steamed solution there was practically no maceration in 48 hours.

The conclusions to be drawn from these data are, first, that pectinase is not produced in Czapek's nutrient solution at any hydrogen-ion concentration tried and, second, that its production in sweet-potato decoction is not interfered with when the hydrogen-ion concentration is not so high as to prevent growth. In other words, pectinase is produced by *R. tritici* when growing in sweet-potato decoction at any hydrogen-ion concentration that will permit its growth. Pectinase was also present in the mycelium.

The amount of sugar present in the control (not inoculated) and the inoculated solutions was determined for Czapek's solution only. At the higher hydrogen-ion concentrations no fungous growth took place and no sugar was used. As a matter of fact, there appears to be a slight increase in the amount of sugar present in the controls over that in the inoculated solutions, probably due to some concentration of the sugars as a result of evaporation. A measurable amount of sugar was consumed by the fungus at all other hydrogen-ion concentrations, the larger amount being at the higher P_H values. It will be noted that the amounts of sugar consumed at the lower hydrogen-ion concentrations are proportionately greater than in the less alkaline solutions. This is also true in the controls. No doubt this variation may be accounted for, at least in part, by the reaction between the sugar and alkali. The largest amount of mycelium was produced at the lower hydrogen-ion concentrations.

An examination of Tables III and IV shows that in Czapek's nutrient solution the amount of mycelium produced increases with the decrease in hydrogen-ion concentration, varying from 0.0283 gm. to 0.1780 gm. (Table III) and from 0.0340 to 0.1734 gm. (Table IV). No mycelium was produced at the highest hydrogen-ion concentration. In experiment 3, the dry weight of the mycelium was not determined, but in experiment 4 (Table VI) there was a more or less gradual increase in dry

material as the hydrogen-ion concentration decreased, with one exception. In the solution with an original P_H of 8.86, which is apparently too alkaline for normal growth, the dry weight was somewhat less than it was in the solution with a P_H of 8.21 (Table VI).

It has been intimated that the maceration which takes place in solutions after they have been steamed is due to the action of some one or more acids produced in the solution by the fungus. A number of acids have been shown by different investigators to be formed by fungi in nutrient solutions, among them being oxalic, acetic, and formic. These three organic acids, together with hydrochloric acid and sodium hydroxid, were made up to different hydrogen-ion concentrations and their ability to dissolve the middle lamellae determined. The results showed that maceration could be brought about by the acids in high hydrogen-ion concentrations and by the alkali at a P_H of about 12 or above. Oxalic acid at a P_H of 2.13 macerated raw sweet-potato disks in from 24 to 48 hours at a P_H of 2.44 in 72 hours. No maceration occurred in 72 hours by this acid at a P_H of 2.76. Acetic acid appeared to require more time to complete maceration than oxalic acid at the same concentration. Acetic acid of P_H 2.13 macerated the disks in 72 hours, while at P_H 2.40 the amount of maceration was very slight in that length of time and was entirely absent at a P_H of 2.44. In a P_H 1.99 of formic acid maceration was nearly complete in 6 hours, at P_H 2.16 in 24 hours, and at P_H 2.23 in 54 hours. At hydrogen-ion concentrations of P_H 2.40 and 2.57, there was slight and no maceration, respectively, in 54 hours by this acid. In full strength formic acid maceration was complete in 3 hours. Hydrochloric acid of a P_H 2 will induce slow maceration. A comparison of these results with those obtained when Czapek's solution was used shows that the loss of coherence of the cells of sweet potato disks occurs in pure solutions of these acids at about the same hydrogen-ion concentration as in Czapek's solution and in about the same length of time. Oxalic acid macerates a little more rapidly than acetic acid at the same concentration, and formic acid a little more rapidly than either oxalic or acetic.

The results of these investigations seem to indicate that no pectinase is produced in Czapek's nutrient solution when glucose is used as a source of carbon. However, an acid is produced in sufficient hydrogen-ion concentration to cause a dissolution of the middle lamellae identical with that caused by pectinase. It was found also that the original P_H value of the solution had no influence on the secretion of pectinase. In all concentrations of Czapek's solution in which the fungus grew the hydrogen-ion concentration was increased, the greatest increase being in the solutions with the highest P_H values.

A middle lamellae splitting enzym was produced when pectin alone was used as a source of carbon in Czapek's nutrient solution and to a less extent when combined with glucose. It had been suspected that the failure to obtain maceration in Czapek's solution was not due to the absence of the enzym, but to its inactivation by the acid formed. Brown (*2*) showed that the addition of some of the organic and mineral acids to extracts of Botrytis mycelium retarded the action of the enzym, the time required to macerate potato disks being increased with the increase in the concentration of the acid. A point was finally reached when inactivation was complete. The retarding action of all the acids, was found to be about the same up to a certain concentration beyond

which the retarding action of the mineral acids increased much more rapidly. A concentration was finally reached where maceration was caused by the acid. Brown in his studies used dilutions of normal acids, so his results can not be compared directly with those of the writers, who studied the action of the acids in terms of hydrogen-ion concentrations. That the acid formed in Czapek's solution did not inactivate the enzym may be inferred from the following facts. When Czapek's solution, which was made acid (P_H 1.8) by the growth of the fungus, was changed to a P_H of 7.8 and raw sweet-potato disks added no maceration took place. It had been previously shown that the enzym was not inactivated at P_H of 10. The solution was not alkaline enough to inactivate the enzym. Some of the data obtained with sweet-potato decoction may be cited as further evidence. It has been shown (Table V) that maceration in sweet-potato decoction is complete in 2¼ hours at a P_H of 2.55. On the other hand, in Czapek's solution with a P_H of 2.66 (Table III) which is even less acid no maceration occurred in 48 hours. Although there is a difference in the composition of these two media, it is reasonable to assume that since the acid was not sufficiently strong in the sweet-potato decoction (P_H 2.55) to inhibit the action of the enzym it would not be able to do so in the less acid Czapek's solution (P_H 2.66).

The investigations of the authors showed that the presence of pectinase in the mycelium grown on Czapek's nutrient solution could not be demonstrated, while it occurred regularly in the hyphae produced on sweet-potato decoction. If the enzym was produced, its occurrence in the hyphae on Czapek's solution as well as on sweet-potato decoction would be expected. Furthermore, the enzym in the mycelium, if it occurred there, would not be inactivated by the acid in the substrate, since the mycelium is always suspended in distilled water, which is practically neutral.

SUMMARY

(1) *Rhizopus tritici*, a fungus capable of causing the softrot of sweet potatoes was used in all these experiments. On suitable media it produces a powerful enzym, called pectinase, which dissolved the middle lamellae of raw sweet-potato and carrot disks.

(2) A comparison of the production of the enzym was made on the following media: String bean, prune, Irish potato, carrot, turnip, and sweet-potato decoction, and on Czapek's, Peffer's, and Richard's synthetic media and on beef bouillon.

(3) It was found that the cell wall dissolving enzym was produced on all the vegetable media except prune decoction, but not on the synthetic media with glucose as a source of carbon or on beef bouillon.

(4) If pectin was used alone as a source of carbon in Czapek's nutrient solution, an active enzym was produced. When pectin was combined with glucose the action on raw sweet-potato disks was feeble.

(5) When *Rhizopus tritici* was grown in Czapek's nutrient solution with glucose as a source of carbon the substrate became sufficiently acid to cause dissolution of the middle lamellae so that coherence of the cells was completely lost in from 6 to 24 hours.

(6) The production of the macerating principle by the fungus when growing on Czapek's nutrient solution and sweet-potato decoction was not influenced by adjusting these solutions to different P_H values. The enzym was not produced in Czapek's solution at any hydrogen-ion

concentration tested. On the other hand, it was secreted in sweet-potato decoction at all concentrations tried.

(7) The best growth as measured by the dry weight of the mycelium took place at the lowest hydrogen-ion concentration.

(8) The amount of glucose consumed in Czapek's solution is correlated with the amount of mycelium produced.

(9) The substrate influences the production of pectinase; the hydrogen-ion concentration does not. The results indicate a well-marked qualitative action of the substrate on the production of the macerating enzym, in that when vegetable decoctions which probably contain pectin are used and when nutrient solutions to which pectin is supplied as a source of carbon is employed pectinase is produced.

LITERATURE CITED

(1) BIERRY, H.
1912. DIE ROLLE DER ELEKTROLYTE BEI DER WIRKUNG EINIGER TIERISCHEN FERMENTE. *In* Biochem. Ztschr., Bd. 40, p. 357–369. Bibliographical footnotes.

(2) BROWN, William.
1915. STUDIES IN THE PHYSIOLOGY OF PARASITISM. I. THE ACTION OF BOTRYTIS CINEREA. *In* Ann. Bot., v. 29, p. 313–348. References, p. 348.

(3) BRUNTON, T. Lauder, and MACFAYDEN, A.
1890. THE FERMENT ACTION OF BACTERIA. *In* Proc. Roy Soc., [London], v. 46, 1889, p. 542–553.

(4) CURRIE, James N.
1917. THE CITRIC ACID FERMENTATION OF ASPERGILLUS NIGER. *In* Jour. Biol. Chem., v. 31, p. 15–37, 2 fig., 2 pl.

(5) DOX, Arthur Wayland.
1910. THE INTERCELLULAR ENZYMES OF PENICILLIUM AND ASPERGILLUS, WITH SPECIAL REFERENCE TO THOSE OF PENICILLIUM CAMEMBERTI. U. S. Dept. Agr. Bur. Anim. Indus. Bul. 120, 70 p. Bibliography, p. 66–70.

(6) DUCLAUX, E.
1899. TRAITÉ DE MICROBIOLOGIE. t. 2. Paris.

(7) GRACE, Linwood G., and HIGHBERGER, Florence.
1920. ACID PRODUCTION BY STREPTOCOCCUS VIRIDANS IN MEDIUMS OF DIFFERENT HYDROGEN-ION CONCENTRATION. *In* Jour. Infect. Diseases, v. 26, p. 451–456.

(8) HARTER, L. L.
1921. AMYLASE OF RHIZOPUS TRITICI, WITH A CONSIDERATION OF ITS SECRETION AND ACTION. *In* Jour. Agr. Research, v. 20, p. 761–786. Literature cited, p. 784–786.

(9) ——— and WEIMER, J. L.
1921. STUDIES IN THE PHYSIOLOGY OF PARASITISM WITH SPECIAL REFERENCE TO THE SECRETION OF PECTINASE BY RHIZOPUS TRITICI. *In* Jour. Agr. Research, v. 21, p. 609–625. Literature cited, p. 624–625.

(10) HUNT, Charles H.
1918. A METHOD FOR PREPARING PECTIN. *In* Science, n. s., v. 48, p. 201–202.

(11) JONES, Horry.
1920. SOME FACTORS INFLUENCING THE FINAL HYDROGEN-ION CONCENTRATION IN BACTERIAL CULTURES WITH SPECIAL REFERENCE TO STREPTOCOCCI. *In* Jour. Infect. Diseases, v. 26, p. 160–164.

(12) KARRER, Joanne L.
1921. STUDIES IN THE PHYSIOLOGY OF THE FUNGI. XIII. THE EFFECT OF HYDROGEN-ION CONCENTRATION UPON THE ACCUMULATION AND ACTIVATION OF AMYLASE PRODUCED BY CERTAIN FUNGI. *In* Ann. Mo. Bot. Gard., v. 8, p. 63–96, 14 fig. Bibliography, p. 95–96.

(13) ——— and WEBB, Robert W.
1920. TITRATION CURVES OF CERTAIN LIQUID CULTURE MEDIA. *In* Ann. Mo. Bot. Gard., v. 7, p. 299–305. Literature, p. 305.

(14) KATZ, J.
1898. DIE REGULATORISCHE BILDUNG VON DIASTASE DURCH PILZE. *In* Jahrb. Wiss. Bot. [Pringsheim], Bd. 31, p. 599–618.

(15) KNUDSON, Lewis.
1913. TANNIC ACID FERMENTATION. *In* Jour. Biol. Chem., v. 14, p. 159–202, 2 fig. Bibliographical footnotes.
(16) KYLIN, Harald.
1914. ÜBER ENZYMBILDUNG UND ENZYMREGULATION BEI EINIGEN SCHIMMELPILZEN. *In* Jahrb. Wiss. Bot. [Pringsheim], Bd. 53, p. 465–501. Literatur-Verzeichnis, p. 500–501.
(17) LAFAR, Franz.
1910. TECHNICAL MYCOLOGY. Transl. by Charles T. C. Salter. v. 2, pt. 2. London.
(18) LIND, K.
1898. UEBER DAS EINDRINGEN VON PILZEN IN KALKGESTEINE UND KNOCHEN. *In* Jahrb. Wiss. Bot. [Pringsheim], v. 32, p. 603–634.
(19) PFEFFER, W.
1896. UEBER REGULATORISCHE BILDUNG VON DIASTASE AUF GRUND DER VON HERRN DR. KATZ IM BOTANISCHEN INSTITUT ANGESTELLTEN UNTERSUCHUNGEN. *In* Ber. Verhand. K. Sächs. Gesell. Wiss. Leipzig, Math. Phys. Cl., Bd. 48, p. 513–518.
(20) WEHMER, C.
1906. CHEMISCHE WIRKUNGEN DER ASPERGILLACEEN. *In* Lafar, Franz. Handb. Tech. Mykol., Aufl. 2, Bd. 4, Lfg. 11, p. 239–270, fig. 77. Literatur, p. 268–270.
(21) WEIMER, J. L., and HARTER, L. L.
1921. GLUCOSE AS A SOURCE OF CARBON FOR CERTAIN SWEET POTATO STORAGE-ROT FUNGI. *In* Jour. Agr. Research, v. 21, p. 189–210. Literature cited, p. 208–210.
(22) WENT, F. A. F. C.
1901. UEBER DEN EINFLUSS DER NAHRUNG AUF DIE ENZYMBILDUNG DURCH MONILIA SITOPHILA (MONT). SACC. *In* Jahrb. Wiss. Bot. [Pringsheim], Bd. 38, p. 611–664.
(23) WOLF, Charles George Lewis, and HARRIS, John Edmund Guy.
1917. CONTRIBUTIONS TO THE BIOCHEMISTRY OF PATHOGENIC ANAEROBES. III. THE EFFECT OF ACIDS ON THE GROWTH OF BACILLUS WELCHII (B. PERFRINGENS) AND BACILLUS SPOROGENES (METCHNIKOFF). *In* Biochem. Jour., v. 11, p. 213–245. References, p. 245.
(24) WORTMANN, Julius.
1882. UNTERSUCHUNGEN ÜBER DAS DIASTATISCHE FERMENTE DER BACTERIEN. *In* Ztschr. Physiol. Chem., Bd. 6, p. 287–329.
(25) YOUNG, H. C., and BENNETT, C. W.
1921. STUDIES IN PARASITISM. I. TOXIC SUBSTANCES PRODUCED BY FUNGI. *In* 22d Ann. Rpt. Mich. Acad. Sci., 1920, p. 205–208. Literature cited, p. 208.
(26) YOUNG, V. H.
1918. SOME FACTORS AFFECTING INULASE FORMATION IN ASPERGILLUS NIGER. *In* Plant World, v. 21, p. 75–87, 114–133. Bibliography, p. 132–133.

THE MICROSCOPIC ESTIMATION OF COLLOIDS IN SOIL SEPARATES[1]

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In a recent work upon soil colloids,[2] in which a description of the soil materials classified as colloids was given, it became desirable to compare the values obtained by an adsorption method for determining the colloidal content of soils with values obtained by some method independent of adsorption phenomena. The most satisfactory method for this purpose would be a mechanical separation of colloidal from noncolloidal soil materials. But it has been shown in previous publications [2,3] that a mechanical analysis probably does not effect a complete separation. However, it seemed possible that colloidal matter not separated from the soil by mechanical analysis might be estimated by microscopical means. Then the sum of the colloid extracted and that estimated microscopically would give a value for the total colloid in the soil by methods quite independent of adsorption.

For the purpose of separating the colloids from the coarser particles of the soils the samples were repeatedly treated by agitation with distilled water containing a trace of ammonia, rubbed with a rubber pestle, and the supernatant liquid decanted and centrifuged. From 40 to 60 such treatments yielded practically all of the colloid extractable by this method. For convenience of manipulation the soils were divided, during the washing and rubbing process, into the colloidal fraction made up of particles less than 0.001 mm. in diameter, a fine fraction made up of particles ranging approximately from 0.001 to 0.050 mm. in diameter, and a coarse fraction consisting of particles larger than about 0.050 mm.[2]

The adsorptive capacities of these fine and coarse residues indicated that they contained colloidal material.[2] Observations of the residues with the ultramicroscope showed that the mineral particles had been fairly well cleaned of adhering colloidal matter by the washing and rubbing, and that the colloidal material remaining in these fractions was in the form of lumps or aggregates made up, at least superficially, of very large numbers of particles less than 0.001 mm. diameter. But since the light coming to the eye from the ultramicroscope is reflected from the surfaces of the particles under examination it was possible that the colloidal aggregates thus observed were simply mineral grains completely coated with colloids or that, at least, the colloidal aggregates

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[2] ANDERSON, M. S., FRY, W. H., GILE, P. L., MIDDLETON, H. E., and ROBINSON, W. O. ABSORPTION BY COLLOIDAL AND NONCOLLOIDAL SOIL CONSTITUENTS. U. S. Dept. Agr. Bul. 1122, 20 p. 1922. Literature cited, p. 19–20.

[3] DAVIS, R. O. E. THE INTERPRETATION OF MECHANICAL ANALYSIS OF SOILS AS AFFECTED BY SOIL COLLOIDS. *In* Jour. Amer. Soc. Agron., v. 14, p. 293–298. 1922. Literature cited, p. 298.

inclosed minute mineral particles. The clean appearance of the obviously mineral particles was, of course, a strong indication that such was not the case, but the element of doubt remained nevertheless.

Under the petrographic microscope in transmitted light these colloidal aggregates were found to be almost universally transparent and, in the rare cases where this was not so, to be highly translucent. Therefore any mineral grain included within the colloidal aggregate would be readily visible provided that either its color or index of refraction was different from the color or index of the colloid. Observation of birefringence between crossed nicol prisms would, of course, readily differentiate between minerals and colloids regardless of similarity of color or refractive index. Such observation, however, showed that practically all of the colloidal aggregates were free from mineral inclusions. Presumably, aggregates containing such had been broken up by the repeated rubbing and washing process.

Therefore, since the residues consist of easily determinable minerals and colloidal aggregates, one readily distinguishable from the other, a microscopic estimation of the relative quantity of each in a given sample was easily obtainable. Owing to the extreme variation of size of particles a straight count would have been subject to large errors. Therefore, the estimation was made by means of a checker-work eyepiece micrometer, relative space occupied by the minerals and colloids being the basis of the calculation. It is believed, on the basis of results obtained with synthetic samples, that errors due to the varying thickness of the particles, both minerals and aggregated colloids, are fairly well balanced and do not appreciably affect the results. Quartz is the predominant mineral in the fractions, and since the specific gravity of this mineral (2.66) and that of the colloids (2.53–2.68) extracted from the soils are very near together, it was believed that differences in specific gravity would not give rise to any serious error.

In order to test this method of microscopic estimation, four synthetic samples were made up, each containing a known amount of colloidal material. Air dried Marshall soil colloid, which had been extracted mechanically and graded by means of the supercentrifuge, was mixed with quartz in the proportion of 1.2 gm. colloid to 0.8 gm. quartz and 0.8 gm. colloid to 1.2 gm. quartz. The first of these mixtures was ground dry to pass a 200-mesh sieve. In order to facilitate any tendency of the colloid to form coatings on the mineral particles, the second sample was ground wet, dried and subsequently rubbed lightly with a pestle to pass a 200-mesh sieve.

Since quartz is practically without cleavage, the particles formed by grinding have a tendency to assume shapes in which the dimensions vary little in different directions within the same particle, although the dimensions of different particles may vary widely. This statement also holds good for the colloidal aggregates. Such a similarity of fracture facilitates a microscopic estimation of the relative quantities of the different constituents present, and therefore does not afford a very difficult test of the method. But, since quartz is the predominant mineral constituent of soils, the results obtained with quartz are probably very near those obtained on the soil separates themselves.

For the purpose of testing the method under more unfavorable conditions, Orangeburg subsoil colloid, extracted mechanically and graded by the supercentrifuge, was mixed with hornblende which has a tendency

to cleave into oblong particles with the greater dimensions several times the magnitude of the smaller, thus presenting a widely different shape from the colloidal aggregates with which it was to be compared. Two samples were prepared consisting of 0.3 gm. colloid to 1.7 gm. hornblende and 0.4 gm. colloid to 1.6 gm. hornblende. The first of these was mixed dry and the second wet, as with the Marshall colloid-quartz samples.

The results of the microscopic examination of these samples are given in Table I.

TABLE I.—*Microscopic estimation of colloids in synthetic samples*

Sample.	Colloid present.	Colloid observed.
	Per cent.	*Per cent.*
Marshall colloid and quartz, mixed dry	60	57
Marshall colloid and quartz, mixed wet	40	45
Orangeburg colloid and hornblende, mixed dry	15	12
Orangeburg colloid and hornblende, mixed wet	20	23

These results showed that the colloid in the fine and coarse residues of the soils could be estimated with fair accuracy by this microscopic method.

Microscopic examinations were therefore made of the fine and coarse residues of eight soils which had been subjected to the repeated washing and rubbing process. The results are given in Table II. The quantity of the unextracted colloid in the fine and coarse residues, as determined by this microscopic method, are given in column 3. The additional data in Table II are given for the purpose of showing the relative amounts of "extractable" and "unextractable" colloids in the soils.

From Table II it will be seen that from 9.4 to 42.4 per cent of the soil was made up of colloids extractable by the methods employed. The microscopic examination of the residues shows that from 25 to 97 per cent of the fine residues and 2 to 25 per cent of the coarse residues were colloidal aggregates not extractable by the methods employed.

These results throw considerable doubt upon the results of several investigators who have determined the colloidal contents of soils and clays by purely mechanical methods. Hissink[4] and Sven Odén,[5] for example, have dispersed colloids in soils by very elaborate washing and rubbing processes. It is of course possible that they may have effected complete dispersion by their methods, but apparently no investigations were undertaken to ascertain whether, or to what extent, aggregates of undispersed colloids remained in their soil suspensions. Williams,[6] in the course of a very elaborate and painstaking separation of colloids from soils by washing, rubbing and boiling, did examine his residues microscopically. Details of his microscopic method are not given, but it is apparent that he used an ordinary chemical microscope and mounted his residues either in air or water. Owing to the wide differences in refractive indices between these mounting media and the ordinary soil minerals, scarcely more than surface phenomena could be

[4] HISSINK, D. J. DIE METHODE DER MECHANISCHEN BODENANALYSE. *In* Internat. Mitt. Bodenkunde, Bd. 11, p. 1–11. 1921.

[5] ODÉN, Sven. ÜBER DIE VORBEHANDLUNG DER BODENPROBEN ZUR MECHANISCHEN ANALYSE. *In* Bul. Geological Inst., Univ. Upsala, v. 16, p. 125–134. 1918–19. Bibliographical footnotes.

[6] WILLIAMS, W. R. UNTERSUCHUNG ÜBER DIE MECHANISCHE BODENANALYSE. *In* Forsch. Geb. Agr.-Phys. (Wollny), Bd. 18, p. 225–350. 1895. Bibliographical footnotes.

observed under these conditions; and consequently the distinction between lumps of aggregated colloids and soil minerals would reduce itself purely to a question of personal judgment based on appearance. Colloidal aggregates might bear very close resemblances to corroded mineral particles and ferruginous or rutilated quartz, for example. Even with a completely equipped petrographic microscope careful cognizance must be taken of the pseudo-similarities of the optics as well as the resemblances of some crystalline and amorphous materials, such as the similarity between colloids subjected to strain and the matted structure of such minerals as chlorite.

TABLE II.—*Microscopic estimation of unextracted colloids in fine and coarse residues of soils*

Sample.	Quantity of fractions separated mechanically, as part of whole soil.	Quantity of unextracted colloid in residues determined microscopically, as part of residue.	Unextracted colloid in combined fine and coarse residues, as part of whole soil.	Unextracted colloid, as part of total colloidal material in soil.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Cecil clay loam, soil:				
Colloid extracted	9.4			
Residue finer particles	7.6	74		
Residue coarser particles	83.8	4	9.0	48.9
Cecil clay loam, soil:				
Colloid extracted	9.4			
Residue finer particles	10.4	55		
Residue coarse particles	78.3	4	8.9	48.6
Huntington loam, soil:				
Colloid extracted	10.3			
Residue finer particles	21.9	48		
Residue coarse particles	64.0	8	15.6	60.2
Huntington loam, subsoil:				
Colloid extracted	13.3			
Residue finer particles	19.4	25		
Residue coarse particles	63.3	10	11.2	45.7
Sassafras silt loam, subsoil:				
Colloid extracted	14.4			
Residue finer particles	20.6	38		
Residue coarse particles	61.9	2	9.1	38.7
Sharkey clay, soil:				
Colloid extracted	42.4			
Residue fine and coarse particles combined	53.1	42	22.3	34.5
Sharkey clay, soil:				
Colloid extracted	31.5			
Residue finer particles	38.9	52		
Residue coarse particles	31.8	25	28.2	47.2
Vega Baja clay loam, soil:				
Colloid extracted	30.5			
Residue fine particles	38.6	97		
Residue coarse particles	31.8	14	41.9	57.9

The calculations given in column 4 of Table II show that from 8.9 to 41.9 per cent of the whole soil was composed of col oidal aggregates not extractable by the methods used. It should be mentioned, however, that the Vega Baja soil which showed a total colloidal content of 72.4

per cent is an exceptional soil. Usually the quantity of colloid present is much lower. Basing the calculations on the colloidal matter alone, it is seen from the table that 34.5 to 60.2 per cent of the total colloids in the soils were not extracted by the methods used. Although this method of repeated rubbing and washing for the extraction of colloids from soils was carried out as carefully and thoroughly as possible, and although we are confident that no more than traces of colloidal material could have been extracted by further washing and rubbing, it by no means follows that other more efficient methods might not be devised.

As practiced in this bureau, microscopic estimation of the amount of colloidal aggregates in the fine and coarse residue is, at best, a long and tedious process. In order to insure fair sampling of the sample under study several mounts must be examined, and several areal counts should be made on each mount. Experience showed that a total of 10 to 12 counts on about four slides gave averages very comparable with those obtained by triple that number of counts on as many more mounts. Nevertheless when only 12 counts are made, the method is too long for routine application.

Unfortunately, the preliminary washing and rubbing are essential in order to remove coatings of the adhering extractable colloid. These coatings are rarely thick enough to interfere with the determination of the mineral; but the microscopic estimation of the quantity of colloid in the coatings would be extremely difficult and inexact. This necessity for washing the soils adds tremendously to the time consumed. Nevertheless, the method is of value in special studies.

SUMMARY

(1) A microscopic method for estimating the quantity of colloidal soil aggregates in soil separates is given.

(2) This method was applied to the residues left after extracting, by repeated rubbing and washing, all colloid possible from eight samples of soil. The results showed that from 34.5 to 60.2 per cent of the total colloidal material in the soil was in the form of colloidal aggregates not extractable by washing and rubbing.

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